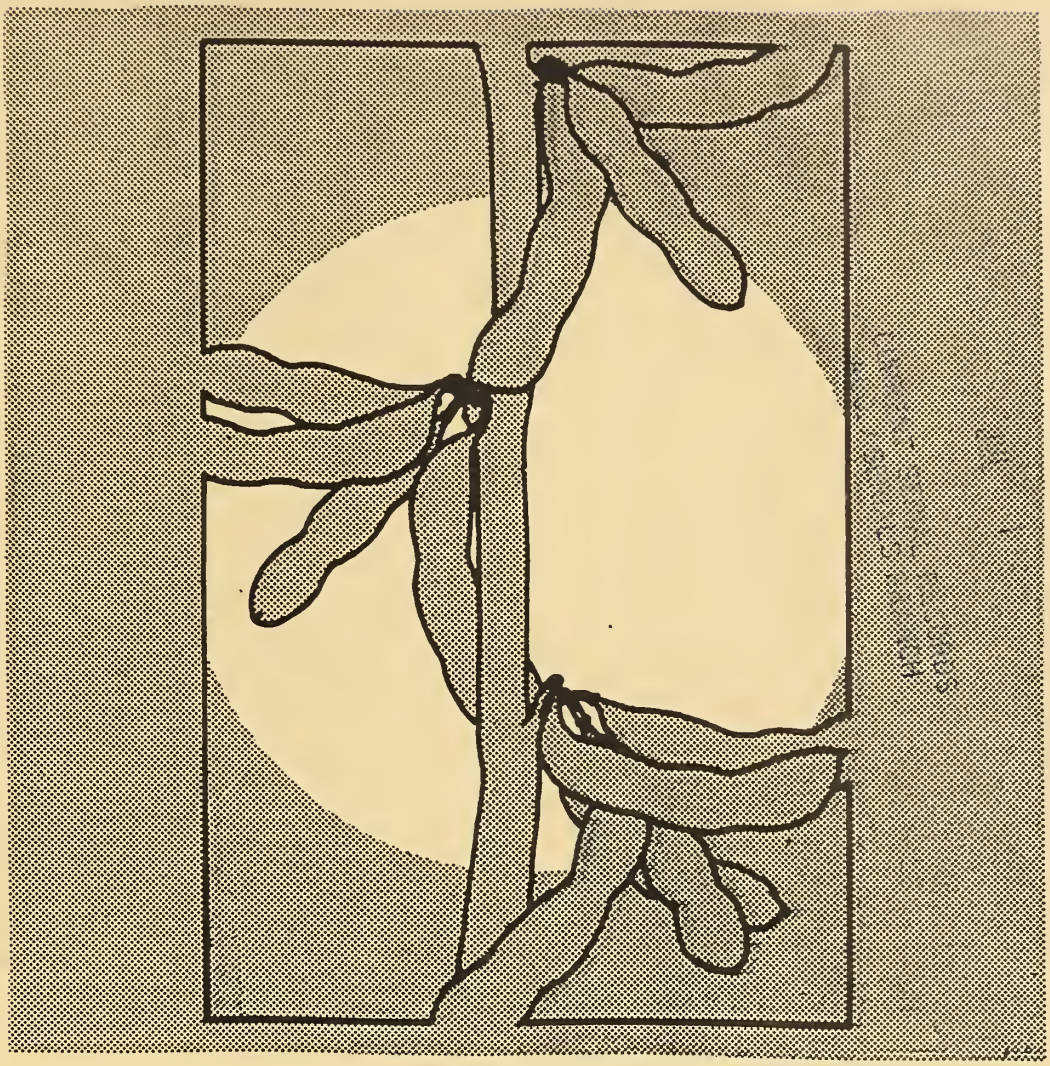
Soybean Genetics Newsletter



Volume 21 May 1994

The data presented here are not to be used in publications without the consent of the respective authors.

USDA-Agricultural Research Service
Department of Agronomy
and Department of Zoology / Genetics
Iowa State University
Ames, Iowa 50011

Table of Contents

UNITED STATES

Foreword

Volume 21, 1994, of the Soybean Genetics Newsletter is impressive both by the quality and quantity of the news notes and articles. The response of individuals, universities, institutions, and agri-business corporations has been most gratifying.

This volume was made possible by the diligent efforts of Arricka Earp with assistance from Ellen Martens. Our sincere appreciation is extended to these two individuals.

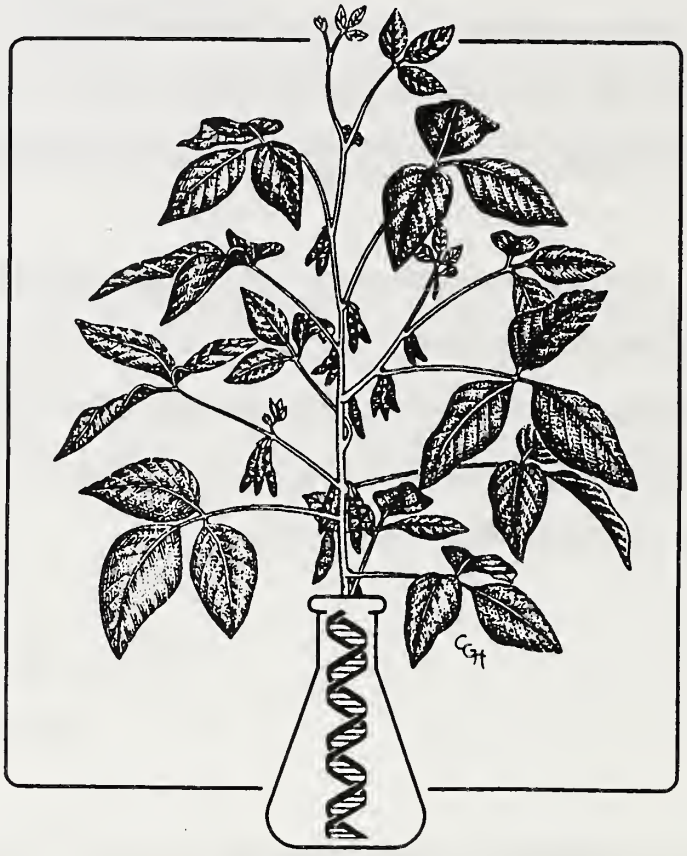
-Reid G. Palmer, editor
USDA- ARS-FCR

Data presented in the Soybean Genetics Newsletter are not to be used in publications without the consent of the respective authors.

Mention of a trademark or proprietary product by the USDA or Iowa State University does not imply its approval to the exclusion of other products that may also be suitable.

5th BIENNIAL CONFERENCE

Molecular & Cellular Biology of the Soybean



July 25—27, 1994
Georgia Center for Continuing Education
Athens, Georgia

The University of Georgia

For information contact:
Center for Soybean Improvement
University of Georgia
3111 Plant Sciences Building
Athens, GA 30602-7272

Soybean Genetics Committee Report

Minutes of meeting held February 16, 1994:

The Soybean Genetics Committee (SGC) met from 5:15 to 6:45 p.m. at the Ramada Convention Center Hotel in Memphis, Tennessee, in conjunction with the National Soybean Breeders' Workshop.

Committee members attending the meeting were: P.B. Cregan, B. Diers, J.H. Orf, H.T. Skorupska, and J.E. Specht. B. Diers and J.H. Orf had been elected by mail ballot to serve a three year term on the Committee. At the conclusion of the meeting , P. Cregan was elected chair for the year ending in February, 1995.

Also in attendance at the meeting were G. Albert, C. Coble, E. Cober, H. Voldeng, and Guodong Zhang. Current Committee members and February expiration dates for their terms on the Committee are:

P.B. Cregan USDA-ARS (1995)
Soybean and Alfalfa Res. Lab
B-011, HH-19, BARC-W
Beltsville, MD 20705
(301) 504-5070
FAX: (301) 504-5728

J.H Orf (1997)
Dept. Agronomy/Plant Genetics
University of Minnesota
St. Paul, MN 55108
(612) 625-8275
FAX: (612) 625-1268

B. Diers (1997)
Dept. of Crop/Soil Sciences
Michigan State University
E. Lansing, MI 48824
(517) 353-4639

R. G. Palmer (ex offico)
USDA-ARS
Dept. Agronomy/Genetics
Iowa State University
Ames, IA 50011
(515) 294-7378
FAX: (515) 294-2299

R.L. Nelson (ex offico)
USDA-ARS, Turner Hall -Agronomy
1102 South Goodwin
University of Illinois
Urbana, IL 61801
(217) 244-4346
FAX: (217) 333-4639

H.T. Skorupska (1996)
Dept. of Agronomy/Soils
Clemson University
Clemson, SC 29634
(803) 656-3523
FAX: (803) 656-3443

C.D. Nickell (1995)
Turner Hall - Agronomy
1102 South Goodwin
University of Illinois
Urbana, IL 61801
(217) 333-9461
FAX: (217) 333-9817

J.E. Specht (1996)
Dept. of Agronomy
Keim Hall, E. Campus
University of Nebraska
Lincoln, NE 68583
(402) 472-1536
FAX: (402) 472-7904

Procedure: As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols, and linkages should be sent to the Chairman of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpolished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then return it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R.L. Nelson is curator for all maturity groups. A form for this purpose immediately follows this article.

Gene symbols will be approved only in cases where the relevant material is available in one of the soybean germplasm collections for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases). The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

Summary of Gene Symbols Approved During the Past Years: One Soybean gene symbol was approved by the Committee during the year ending February 1994 and is given in Table 1.

Table 1. Gene symbols approved March, 1993- February, 1994

Date	Authors	Trait	Gene/Linkage
December 6, 1993	Luzzi, Boerma and Hussey	Resistance to Southern Root-knot Nematode	*Rmi*1

Committee Actions: At the 1993 meeting of the SGC, a committee of J.E. Specht (Chair) and R.L. Nelson was formed to investigate how possible future restrictions (through material transfer agreements) on genes that are approved by the SGC will affect future gene symbol assignments. As part of this investigation, J.E. Specht conducted a survey of soybean breeders and geneticists to assess their opinions on current SGC policy regarding the requirement of allelism tests with previously named genes conditioning similar phenotypes. The survey questions and a summary of the responses as reported to the SGC by J.E. Specht are given below:

1. Item #4 of the "Guidelines on Evidence Necessary for the Assignment of Gene Symbols" states that, "For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes."

Do you feel that Item #4 of the guidelines should be retained by the SGC as a requirement for SGC approval of a gene symbol for a newly discovered gene?

Yes: 27 **Maybe:** 1

2. Do you agree with the published SGC policy of approving gene symbols only in cases where the relevant material is made available (for allelism tests)?

Yes: 26 **No:** 1 **Maybe:** 1

3. Do you feel that the SGC should revise the two former rules to better conform with the "prevailing norm" of germplasm use agreements?

Yes: 5 **No:** 21 **Maybe:** 2

4. Do you feel that, if a standard allelism test is adopted by all parties, the form should include the following:

a) requestor agrees to use the requested germplasm solely for an allelism test?

Yes: 25 **No:** 2 **Maybe:** 1

b) requestor agrees to destroy all requested germplasm and its descendant progeny upon completion of the allelism test?

Yes: 24 **No:** 4

c) requestor agrees to inform the supplier of the allelism tests as soon as they are available?

Yes: 20 **No:** 8

d) supplier agrees that requestor is free to publish or disclose the allelism test information?

Yes: 25 **No:** 2 **Maybe:** 1

As a consequence of the survey responses and subsequent discussion, the SGC unanimously voted to amend the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols" by appending the following statement to Guideline #4:

"If appropriate allelism tests are not included in a manuscript the committee will request such information from the researcher before gene symbol approval."

The SGC unanimously voted to reiterate the policy of the Committee that was stated in the Soybean Genetics Newsletter, Vol. 14 p. 6, 1987:

"Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing."

The Registration of Soybean Mapping Populations: The SGC unanimously voted to recommend the following policy to Crop Science and to the USDA:

"That soybean populations used for genetic mapping be registered in Crop Science with the understanding that the registering institution maintain the population for five years after which the Soybean Crop Advisory Committee will determine if the population will be maintained by the USDA Soybean Germplasm Collection."

Drs. J.E. Specht and J.H. Orf will forward this recommendation to Crop Science and Dr. P.B. Cregan will determine the appropriate USDA ARS official with

whom to communicate, relative to the maintenance of populations by the USDA Soybean Germplasm Collection.

Consideration of a New Category of "Non-distributable" seed by the National Seed Storage Lab (NSSL): A seed sample of soybean and other crop cultivars receiving Plant Variety Protection Certificates from the Plant Variety Protection Office of the USDA, Agricultural Marketing Service is currently stored at the NSSL. The NSSL is considering broadening the seed materials that will be held under the designation of "Non-distributable." If this category is broadened, the SGC unanimously recommended that:

"If a new non-distributable category is established by the National Seed Storage Laboratory, authors receiving SGC gene symbol approval will provide a seed sample of the appropriate genetic material to the NSSL."

P.B Cregan
Chair

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____

Submitted by: _____

Address: _____

_____

_____

_____

_____

Strain Designation: _____

Genotype: _____

T number (assigned by curator):

Return to:

R. L. Nelson, curator

USDA Soybean Germplasm Collection

Department of Agronomy

University of Illinois

1102 South Goodwin Avenue

Urbana, Illinois, 61801 U.S.A.

Phenotype: _____

_____

(List the gene(s) and a description of the phenotype of the trait)

Parental Origin: _____

_____

_____

When and where found and by whom: _____

_____

_____

(Include year, location, institution, and name of individual making find or in charge of research).

Description: Maturity Group ____ Stem termination _____ Flower color _____

Pubescence color _____ Pubescence type and density _____ Pod color _____

Seed coat luster and color _____ Hilum color _____ Other _____

Special instructions for growing or maintenance, if any: _____

_____

Literature Reference: _____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.)

Date seedlot received at Urbana: _____ Date T number assigned: _____

USDA-AGRICULTURAL RESEARCH SERVICE
National Soybean Research Laboratory
1101 W. Peabody Drive
Urbana, Illinois 61801

USDA SOYBEAN GERMPLASM COLLECTION REPORT

February 1994

In 1993, a total of 13,314 seedlots were distributed from the USDA Soybean Germplasm Collection in response to 342 requests from 37 states and 22 foreign countries. There were 295 domestic seed orders for 12, 540 seedlots and 47 foreign requests for 889 seedlots. Numerous publications were sent in response to 11 domestic and 6 foreign requests for information about the collection. Additionally, 342 accessions were sent to the National Seed Storage Laboratory at Ft. Collins, Colorado, as back-up samples for the collection.

Of the approximately 13,500 Glycine max strains in the collection in 1993, 1600 were grown in 4-row plots at Urbana, Illinois, and 135 were grown in 4-row plots at Stoneville, Mississippi, for seed replacement. An additional 96 group X accessions were planted in 12-foot, single-row plots in Puerto Rico for seed replacement in November 1993. These lines will be harvested in March 1994 and will then be available for distribution.

Of the 3424 pureline and comparison plant rows grown in 1993, 568 were grown at Stoneville and 2856 were grown at Urbana. Comparison plant rows are those of an established strain which is grown alongside the pureline plant rows when no conclusion is reached about the equality of a new accession and an established strain the first year an accession is grown. The total number of G. max accessions added to the collection this year was nearly 1000, with purelines added from China, Russia, Nepal, South Korea, Japan and Taiwan. Included in this number are the 737 purelines harvested from the 500 Chinese accessions received in May of 1992. Many of these original seedlots were mixed. The final number of lines added to the collection may be slightly lower after all of this year's seed samples have been compared.

In Puerto Rico, single plants for purelining were harvested in February 1993 from the 153 maturity group IX accessions in the collection plus 194 new accessions from Indonesia and 5 new accessions from China. Seed production was extremely poor for many of these plants. Therefore, seed of 232 of these were planted in November 1993, in 5-foot, single-row plots for seed increase. These

lines will be harvested sometime this month and will be planted by the end of March in 8-foot rows, along with the remaining maturity group IX accessions. The purelining and evaluation of this group will be completed this year, and seed of these lines will again be available for distribution sometime this year. Three years experience with growing the group IX's and X's indicates that when planted in Puerto Rico, group IX's are best grown in the summer and group X's in winter.

Accessions grown for the first time in 1993 originated from China, Russia, Vietnam, Argentina and Japan. Of these, 148 G. max accessions were grown at Urbana and 81 were grown at Stoneville. The following institutions donated this germplasm: Japanese National Federation of Agriculture Cooperative Association, Tokyo, Japan; Organizacion Ferrarotti para el Campo, Buenos Aries, Argentina; National Scientific Committee, Beijing, China; Institute of Crop Germplasm Resources, Beijing, China; University of Can Tho, Department of Genetics and Plant Breeding, Can Tho, Vietnam. The following people helped to obtain these new accessions and their assistance is greatly appreciated: R.L. Bernard, University of Illinois; Y. Chen, Institute of Crop Germplasm Resources; J.S. Ferrarotti, Organizacion Ferratotti para el Campo; K. Naito, Japanese National Federation of Agriculture Cooperative Association; T.T. VanToai, USDA-ARS; T.D. Vuong, University of Can Tho; G. White, USDA-ARS-PSI-NGRL-PIO. We would also like to thank the Illinois Soybean Program Operating Board, Iowa Soybean Promotion Board, Iowa Agriculture and Home Economics Experiment Station, Illinois Agricultural Experiment Station and USDA-ARS for their support of the Chinese germplasm exchange project.

Dr. Qiu Lijuan, the visiting scholar from China currently working with the germplasm project, is characterizing some of their ancestors of the modern Chinese cultivars. She will be comparing them with the ancestors of modern U.S. cultivars, most of which originated in China. Several of the ancestors of the modern Chinese lines were already in our collection, but Dr. Qiu identified 20 that were not. These lines were requested from the Institute of Crop Germplasm Resources, Beijing, China and have been received at Urbana. In addition to the 20 ancestral lines from China, which will be planted in 1994, 20 G. max accessions have been received from Japan, seven from the Russian Federation and one from India. No new accessions of G. soja have been received for planting in 1994.

Sixty-five plots of G. Soja were grown at Urbana and 9 plots were grown at Stoneville for seed increase in 1993. Four new lines, all from Russia, were added to the wild soybean collection this year, bringing the current inventory of the USDA

Wild Soybean Germplasm Collection to 1040 accessions. Grown for the first time this year at Urbana were 22 accessions from eastern Russia, which were donated by the Far Eastern Experiment Station, All-Russian Institute of Plants, Vladivostok, Russia, and the Lenin All-Union Academy of Agricultural Sciences, Far East Department, All-Russian Institute of Plant.s, Blagoveshchensk, Russia. This germplasm was obtained by T.A. Lumkin, Washington State University

Unpublished data for accessions in maturity groups less than V which were introduced before 1963 have been submitted for publication in a USDA Technical Bulletin. This information had been available previously as locally produced bulletins, but was never released in a formal publication. These data were revised so that the format of this publication would follow that of previously published technical bulletins containing general evaluation data. Pubescence form and density data have been added; reported stem termination codes are based upon the stem termination score; and the country of origin was reviewed and updated, when more current information was available. Once we receive copies, this publication will be distributed to soybean researchers throughout the world. We plan to include updated versions of non-published information about the soybean collection in this mailing. In 1993, the general evaluation of 812 accessions of maturity group VI was completed at Stoneville. These data will be summarized and published in a USDA Technical Bulletin this year. This spring, we will begin the evaluation of approximately 760 accessions of maturity group VII and VIII at Stoneville. Also about 800 accessions of maturity groups less than V, which have been added to the collection since 1987, will undergo the first year of general evaluation at Urbana in 1994.

Of the 414 maturity group V G. max accessions evaluated at Stoneville for reaction to stem canker, 131 lines were found to be highly resistant. Additionally, 880 maturity group VIII , IX and X G. max lines were evaluated for resistance to leaf feeding by velvet bean caterpillar, but none were identified as being highly resistant. Evaluation of 1,940 G. max and 570 G. soja germplasm accessions for resistance to races 3,5, and 14 of the soybean cyst nematode was conducted at Jackson, Tennessee. Of the G. max lines, 22 were resistant to at least one race, 10 were moderately resistant to at least one race and PI 467312 was resistant to all three races. Initial results indicate that eight accessions of G. soja are resistant to race 3, four are resistant to race 5 and three are resistant to race 14. Additional evaluation is being conducted to confirm these results.

Data from S.C. Anand's soybean cyst nematode screening study and T.C. Kilen's stem canker study have been received and will be added to the GRIN database with the assistance of members of the Database Management Unit in Beltsville, Maryland. Also to be added to the GRIN database is the information being gathered about the newly added Chinese lines. These data will be entered into the GRIN as soon as possible. The re-designed and updated version of GRIN is scheduled to be up and running in late June of 1994.

C. J. Coble
R.L. Nelson

SOYBEAN INSTITUTE
Jilin Academy of Agricultural Sciences
Gongzhuling, 136100
Jilin Province
Peoples Republic of China

Polymorphism and geographical distribution of fat content of wild soybean *G. soja* in China.

Soybean, the most important crop containing both protein and fat in the world, originated in China. It is widely accepted that the wild soybean G. soja is the ancestor of cultivated soybean G. max. There have been more than 5000 wild soybean germplasms have been collected in China. It is about 90% of the : collections all over the world. The purpose of this study was to analyze the polymorphism and geographical distribution of fat content of 5147 wild soybean germplasms with different evolutionary levels, in order . to provide guidelines to apply fat of wild soybean, and to provide information on the evolution and origination of soybean.

Materials and Methods: According to "Catalogue of Chinese wild soybean germplasms", the longitudes, the latitudes for each germplasm was looked up. Data of all characters were put into micro-computer and data base was established using dBASE III (in Chinese). We analyzed the main fat content, variation coefficient of the germplasms with different seed sizes, from different latitude, different longitude and different geographical regions (each two latitudes and three longitudes form a region). Furthermore, all the germplasms were divided into three types, as wild type (100 seed weight < 2.5 g), semi-wild type I (100 seed weight 2.51-5.0 g), and semi-wild type II (100 seed weight > 5.0 g.). Fat content of different types was compared.

Results and Discussion:

1. Relationship of 100 seed weight and fat content: Fat content of those whose 100 seed weight lower than 2.5g is usually lower than 11%. It is about 14% for those whose 100 seed weight is 5g and about 16% for those whose 100 seed weight is 10g. The correlation coefficient of 100 seed weight and fat content is 0.9404**. The variation coefficient showed that the materials with 100 seed weighing less than 5g was greater than that of the materials with 100 seed weight above 5g.

One hundred seed weight is a major evolutionary character in subgenus Soja. Wang Junling pointed out that 100 seed weight was the most important evolutionary character in soybean. Other researchers also found the same relationships between those two characters. Significant positive correlation was found between fat content and 100 seed weight in wild soybean. Therefore, fat content may be regarded as a important evolutionary character.

It is accepted that subgenus Soja can be classified into wild (G. soja) and cultivated soybean (G, max) species. Someone proposed that another species might be considered between the above two species based on the presence of some intermediate type. This may be resulted from artificial and natural selections. Nearly 100 intermedium types were discovered all over the country since 1970. These germplasms were widely used in physiological, biochemical, genetics and breeding studies. In this research, the intermediate type was usually called semi-wild, or semi-cultivated soybean (G. gracilis). We proposed that the wild soybean should be grouped into typical wild, semi-wild I and semi-wild II, three categories based on 100 seed weight. The fat content of wild soybean all over the country was analyzed based on this standard. The results showed that significant differences existed among these groups. This can be as a reference in evolutionary classification of subgenus Soja.

Table 1. Fat content of different wild soybean groups.

Groups	No. of accessions	100 seed weight (g)	Fat content	
			Mean (%)	C.V. (%)
Wild	4162	1.49	9.23	18.60
Semiwild I	542	3.78	13.01	18.10
Semiwild II	443	7.03	15.23	12.60

2 Geographical distribution of wild soybean according to fat content in China.

A.) Relationship between fat content and longitude: significant negative correlation existed between fat content and longitude (-0.8862**). Fat content decrease with longitude gradually increased from 12.50% at 97E to 7.70% at 134E. The germplasm with high fat content mainly distributed on the west of 115E, inland of China. While those with low fat content are mainly distributed in the Northeast in China.

B.) Relationship between fat content and latitude: The relations between fat content and latitude can be described with cubic curve for wild type. Germplasms with high fat content are mainly distributed between 34N and 39N and the highest content appears at 38N. The germplasm with low fat content usually distributed over the middle and the north part of Northeast (43N-50N) and Southern China (26N-28N).

C.) Relationship between fat content and geographical regions: Germplasms with high far content distributed on plateau region in Northwest. The average fat content of region 38-39NX104-106E and 38-39NX107-109E were 15.63% and 13.14% respectively, while germplasms with lowest fat content tended to decrease from west to east between 38N and 39N.

Obviously, altitude, as well as the natural conditions in western China are favorite for high fat content. Lower temperature, larger diurnal temperature differences and stronger radiation are the meteorological characters of high altitude region. The northeast part of the plateau is characterized with less precipitation, lower humidity. While that of the southwest part of the plateau is characterized with high temperature, more precipitation and higher humidity. The average fat content of 154 wild germplasms collected from high altitude region (> 1000m) as 10.15% , that of 2892 accessions collected from low altitude (<400m) was 9.00%. These data indicated that the natural condition at high altitude is favorable to fat formation. Because wild soybean are not affected by cultivation so the relationships between fat content and natural conditions are more objective.

3. Comparisons among wild, semi-wild I and semi-wild II.

A.) Fat content of semi-wild I was higher than wild soybean, and that of semi-wild II was higher than semi-wild I.

B.) Fat content of wild soybean tally with mono-peak curve, and that of semi-wild type I and semi-wild type II tally with cubic curve. the two high peak regions were located on 34-37N and 42-47N, while the low peak region was located on 38-40N. Closest region between wild and semi-wild wild soybean, the closest region of fat content may be related to soybean original region

We have ever studied and compared some bioecological and biochemical characters between wild and cultivated soybeans. We found some characters were very similar between these two species in the region of Yellow River Valley. The

region where the fat content between wild and semi-wild types was closest is just at Yellow River region. This may be as a reference of soybean evolutionary study..

References:

Shu Shizhen. 1986. A preliminary study on the evolution of the main characteristics of soybean. Acta Agronomica Sinica 12(4):255-259.

Wang Jinling. 1986. Effect of backcrossing on overcoming viny and lodging habit of cultivated X wild and cultivated X semi-wild crosses. Soybean Science 5(3):181-187.

Xu Bao, Zheng Huiyu, Lu Qinhua, Zhao Shuwen & Zhou Shuhua & Hu Zhiang. 1986. Three new evidences of the original area of soybean. Soybean Science 5(2):123-130.

Xu Bao & Lu Quinhua. 1988. Comparative study on flowering and podding characteristics of wild, semiwild, semicultivated and cultivated soybean. Soybean Science 7(2):103-112.

Xu Bao. 1989. A decade of wild soybean research in China. Proceedings of World Soybean Research Conference IV. p. 104-111.

Xu Bao, Zhuang Bingchang, Lu Qinhua & Lu Jingliang. 1989. Study on the superoxide dismutase of soybean seeds from different species in subgenus soja. Acta Botanica Sinica 31(7):517-522.

Zhuang Bingchang
Xu Hang
Wang Yumin
Li Fushan
Xu Bao

* This project is funded by NNSF

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, Jiangsu 210014
The People's Republic of China

A study on genotypic variation of tofu yield, quality and processing traits of soybean landraces

Tofu, a traditional food in east Asia,especially in China, has been getting popular in western countries. Accordingly, breeding for tofu yield and quality has become an important issue concerned by both breeders and consummers. Great diversity of economic traits appeared among soybean landraces developed by farmers throughout Chinese history. The present paper was to study the genotypic variation of tofu yield, quality and processing traits among landraces.

Materials and Methods: A sample of 210 landraces from Huang-Huai-Hai valleys and southern China were tested in a randomized complete block design with two replications in Nanjing in 1992. The yield, quality and processing traits of tofu were measured. Tofu yield traits include: dried tofu yield and fresh tofu yield. Tofu quality traits include: protein content in dried tofu, oil content in dried tofu, total content of protein and oil in dried tofu, as well as amount of protein in tofu , amount of oil in tofu, total amount of protein and oil in tofu. The tofu processing traits include recovery of protein, recovery of oil, extractablity of protein, coagulability of protein, residue rate, protein content in residue and oil content in residue.

Results: The average and standard deviation of dried tofu yield from 100g dried seeds among landraces was 50.0±5.2g and that of fresh tofu was 246.1±51.7g (Table 1). The genotypic coefficients of variation of the dried and fresh tofu yield were 10.31% and 19.78% and the heritabilities were 94.43% and 75.18%, respectively. It indicated that the genotypic variation of tofu yield among landraces were abundant and that the gene resources of high tofu yield could be found.

The 100g dried seeds produced tofu protein 16.2g (6.9-26.1g), tofu oil 11.3g(3.1-15.1g) , in a total of 27.5g (11.9-41.9g). The content of protein, oil and both of them in dried tofu were 32.3% (15.0-48.2%), 22.9% (10.1-27.0%) and 55.2% (38.5%-71.8%), respectively, which suggested that breeding for tofu yield needed both high protein content parents and high oil content parents. In addition, the C.V. values of the six tofu

quality traits, the amount of protein in tofu and protein content in dried tofu in particular, were all quite large (13.55%-30.89%), which indicated that there existed great selection potential for each of the six traits.

Because the recovery of protein (35.5%, 14.8%-59.2%) was much less than the recovery of oil (80.2%, 28.0%-95.1%), the tofu breeding should especially emphasize the improvement of the recovery of protein. There also existed large amount of variation and selection potential of the recovery of protein, the extractability of protein and the coagulability of protein. In addition, the large residue rate (37.2%, 27.0%-60.5%) indicated also the necessity of improvement of it through both breeding and processing approach. Ten superior landraces of dried tofu yield, ten of fresh tofu yield, and five of each quality and processing traits, respectively, were screened out for breeding program of tofu processing. The differences from the overall mean were listed in Table 2. In addition, one landrace with fragrant flavor of fresh tofu was found.

Table 1. Variability of tofu yield, quality and processing traits among landraces of soybean.

Trait	$\bar{X}$	S	X_{min}-X_{max}	C.V. (%)
Dried tofu yield	50.0	5.2	31.2-59.8	10.3
Fresh tofu yield	246.1	51.7	155.7-403.5	19.7
Protein content in tofu	32.3	8.4	15.0-48.2	26.0
Oil content in tofu	22.9	3.1	10.1-27.0	13.5
Content of protein and oil in tofu	55.2	9.3	38.5-71.8	16.8
Amount of protein in tofu	16.2	1.9	6.9-26.1	30.8
Amount of oil in tofu	11.3	5.0	3.1-15.1	16.9
Amount of protein and oil in tofu	27.5	6.1	11.9-41.1	22.2
Recovery of protein	35.5	11.0	14.8-59.2	31.1
Recovery of oil	180.2	11.7	28.0-95.1	14.6
Extractability of protein	65.6	11.7	44.7-88.5	11.8
Coagulability of protein	55.0	16.7	18.9-97.1	30.4
Residue rate	37.2	5.3	27.0-60.5	14.2
Protein content in residue	28.5	3.7	20.9-35.1	12.9
Oil content in residue	14.0	2.5	8.2-18.6	17.9

Table 2. Superior landraces selected for tofu yield, quality and processing traits.

Trait	n	X-$\bar{X}$
Dried tofu yield	10	6.8-9.8
Fresh tofu yield	10	86.2-139.5
Protein content in tofu	5	13.0-15.9
Oil content in tofu	5	3.4-4.1
Content of protein and oil in tofu	5	12.8-16.6
Amount of protein in tofu	5	7.7-7.9
Amount of oil in tofu	5	2.0-3.8
Amount of protein and oil in tofu	5	8.9-13.6
Recovery of protein	5	16.7-22.3
Recovery of oil	5	12.0-14.9
Extractability of protein	5	17.5-23.5
Coagulability of protein	5	22.1-42.1
Residue rate	5	5.9-10.2
Protein content in residue	5	5.4-7.6
Oil content in residue	5	4.0-5.8

References:

Johnson, L.D. AND L.A. Wilson, 1984, Influence of soybean variety and the method of processing in tofu manufacturing: comparison of methods for measuring soluable solid in soymilk, J. Food Sci., 49:202-204.

Lim, B.T., et al., 1990, Yield and quality of tofu as affected by soybean and soy milk characteristics, calcium sulfate coagulant, J.Food Sci., 55 (4):1088-1092.

Wang, H.L., 1983, Effect of soybean varieties on the yield and quality of tofu, Cereal Chem., 60(3):245-248.

Wang H.L. and J.F.Cavins, 1989, Yield and amino acid composition of fractions obtained during tofu production, Cereal Chem., 66(5):359-361.

Zhou,X.A., J.Y.Gai and Y.H.Ma, 1992, Variation of tofu processing traits and their relationship with components of seed storage protein in soybean, Soybean Science, 11(4):283-289.

Zhang, X.B., 1992, A preliminary study on genetic variation of tofu processing characters of soybean landraces, Master thesis , Nanjing Agricultural University Library.

Junyi Gai

Junpei Jin

Zhanglin Cui

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, Jiangsu 210014
The People's Republic of China

Correlation analysis regarding tofu yield, quality and processing traits of soybean landraces

Tofu yield and quality have been studied as important breeding objectives since 1970s'. It has been reported that the tofu yield is correlated with protein content in seed, content of components of storage protein in seed, oil content in seed,content of soluable solid in soymilk, ash element content and vitamine content.There were also some studies on correlations regarding the tofu quality traits such as tofu protein content,tofu hardness, amino acid composition and vitamine content. An attempt of our present work was to study the correlations on tofu yield, quality and processing traits in order to provide some information for breeding methodology.

Materials and methods: A sample of 210 soybean landraces from Huang-Huai-Hai valleys and sourthern China were tested in a randomized complete-block design with two replications in Nanjing in 1992. The yield, quality and processing traits of tofu were measured. Tofu yield traits include dried tofu yield and the fresh tofu yield. Tofu quality traitsinclude protein content in dried tofu, oil content in dried tofu, total content of protein and oil in dried tofu, amount of protein in tofu, amount of oil in tofu, total content of protein and oil in tofu. The tofu processing traits include recovery of protein, recovery of oil, extractablity of protein, coagulability of protein, residue rate, protein content in residue and oil content in residue.

Results: The tofu yield was positively correlated with recovery of oil and that of protein, and negatively correlated with residue rate, protein content and oil content in residue (Table 1) . These results indicated that tofu yield was influenced by both recovery of protein and recovery of oil, and that tofu yield could be improved by selecting parents with low residue rate. Stepwise multiple regression analysis indicated that the dried tofu yield was related with recovery of oil, recovery of protein, oil content in seed and protein content in seed while the fresh tofu yield was related with recovery of protein, residue rate, 100-seed weight and the amount of protein in soymilk. There appeared positive correlation almost between all pairs of the quality traits. And all tofu qulity traits were positively correlated with the recovery of protein , that of oil, and coagulability of protein (Table2). Among the processing traits, coagulability was correlated with almost the others(Table 3).

Table 1. Correlation between tofu yield and other traits

Trait	Days to flowering	Days to maturity	100- seed wt.	Fresh tofu yield	Oil content	Protein content`	Extract-ability
Dried tofu yield	0.156*	0.166*	0.386**	0.748**	-0.150*	0.194*	0.173
Fresh tofu yield	0.070	0.085	0.0258**		-0.169*	0.216**	0.163

Trait	Coagulanility of protein	Recovery of protein	Recovery of oil	Residue rate	Protein content in residue	Oil content in residue
Dried tofu yield	0.284*	0.531**	0.729**	-0.475**	-0.461**	-2.78**
Fresh tofu yield	-0.100	0.494**	0.346**	-0.244	-0.214	-0.502**

Note: * and ** represent significant at 0.05 and 0.01 level, respectively.

Table 2. Correlations regarding tofu quality traits.

Trait	Oil content in tofu	Total content of oil and protein in tofu	Amount of protein in tofu	Amount of oil in tofu	Amount of protein and oil in tofu
Protein content in tofu	0.109	0.944**	0.943**	0.260	0.854**
Oil content in tofu		0.430**	0.132	0.787**	0.354**
Content of protein and oil in tofu			0.900**	0.497**	0.893**
Amount of protein in tofu				0.452**	0.960**
Amount of oil in tofu					0.683**

Traitt	Extractability of protein	Coagulability of protein	Recovery of protein	Recovery of oil	Residue rate	Protein content in tofu	Oil content in tofu
Protein content in tofu	0.240	0.768**	0.940**	0.213	-0.178	-0.164	0.308*
Oil content in tofu	0.200	0.162	0.234	0.405**	-0.083	-0.454**	0.262
Content of protein and oil in tofu	0.284*	0.751**	0.932**	0.328*	-0.189	-.299*	0.366**
Amount of protein in tofu	0.314*	0.779**	0.983**	0.418**	-0.227	-0.174	0.281*
Amount of oil in tofu	0.338*	0.352*	0.506**	0.704**	-0.170	-0.431**	0.244
Amount of protein and oil in tofu	0.363**	0.748**	0.963**	0.562**	-0.239	-0.277*	0.307*

Trait	100- seed weight	Days to flowering	Days to maturity	Dired tofu yield	Fresh tofu yield	Oil content	Protein content
Protein content in tofu	0.122	-0.038	0.002	0.274*	0.363**	0.054	-0.002
Oil content in tofu	0.094	-0.410**	-0.339*	0.139	-0.022	0.647**	-0.638**
Content of protein and oil in tofu	0.142	-0.170	-0.110	0.295*	0.322*	0.263	-0.213
Amount of protein in tofu	0.235	-0.0421	-0.001	0.568**	0.536**	0.083	0.059
Amount of oil in tofu	0.279*	-0.347*	-0.273*	0.703**	0.371**	0.556**	-0.358**
Amount of protein and oil in tofu	0.280*	-0.143	-0.086	0.685**	0.555**	0.242	-0.064

Table 3. Correlation among tofu processing traits.

Trait	Recovery of oil	Extractability of prortein	Coagulability of protein	Residue rate	Protein content in residue	Oil content in residue
Recovery of protein	0.412**	0.344**	0.777**	-0.218	-0.243	0.330*
Recovery of oil		0.208	0.358**	-0.143	-0.145	0.137
Extractability of protein			-0.299*	0.125	-0.176	0.048
Coagulability of protein				-0.302*	-0.160	0.312*
Residue rate					0.344*	0.392*
Protein content in residue						-0.013

References:

Lim, B.T., et al. 1990. Yield and quality of tofu as affected by soybean and soy milk characteristics, calcium sulfate coagulant, J. Food Sci., 55 (4):1088-1092.

Zhou, X.A., J.Y. Gai and Y.H. Ma. 1992. Variation of tofu processing traits and their relationships with components of seed storage protein in soybean, Soybean Science, 11(4):283-289.

Zhang, X.B. 1992. A preliminary study on genetic variation of tofu processing characters of soybean landraces, Master thesis, Nanjing Agricultural University Library.

Junyi Gai

Junpei Jin

Zhong Gao

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, Jiangsu 210014
The People's Republic of China

A Study on Genotypic Variation of Components of Storage Protein of Soybean Landraces

The storage protein in soybean seeds can be classified into four major components: globulin, albumin, prolamin and glutelin. The present paper studied the genotypic variation of components of storage protein of soybean landraces and their relationship with tofu yield,quality and processing traits.

Materials and Methods: A sample of 210 soybean landraces from Huang-Huai-Hai valleys and southern China were tested in a randomized complete-block design with two replications in Nanjing in 1992. The yield, quality and processing traits of tofu were measured. Tofu yield traits included dried tofu yield and fresh tofu yield. Tofu quality traits include protein content in dried tofu, oil content in dried tofu, total content of protein and oil in dried tofu, amount of protein in tofu, amount of oil in tofu, total amount of protein and oil in tofu. The tofu processing traits include recovery of protein, recovery of oil, extractability of protein, coagulability of protein, residue rate, protein content in residue and oil content in residue. The testing of storage protein components was conducted according to Kapoor (1970).

Results: There existed great genotypic variation in component of storage protein among soybean landraces (Table 1). The content of globulin in soybean seeds, the highest among four components, was about 31.11% (23.10-38.80%), that of albumin was 2.55% (1.30-5.00%), that of prolamin was 2.26% (0.55-6.40%), and that of glutelin was 5.88% (1.90 -13.35%) (Table 1 and 2). There existed abundant genotypic variation and selection potential of four components. There were only a few significant correlations among the four components, and between the components and tofu yield, quality and processing traits, but not large enough (Table 3), which was different from Zhou et al. (1992) . Superior landraces with high globulin, albumin, prolamin and glutelin were screened out, respectively, for breeding purposes. Under 5% selection pressure,10

landraces were selected for each of high globulin, albumin, prolamin and glutelin content respectively. The contents of globulin, albumin, prolamin and glutelin in landraces selected were 5.49-7.69%, 1.40-2.45%, 1.99-7.34% and 3.89-7.57% higher than mean values of all landraces, respectively. The content of Met and Try in prolamin and that of Met in albumin were relatively high, so to raise the content of albumin and prolamin could increase the Met and Try content in soybean seed such that the quality of amino acid composition could be improved. As shown in Table 2, the genotypic coefficients of variation of prolamin and albumin were 25.96% and 41.11% respectively, which indicated that there existed great selection potential.

References:

Chen, X., 1989, Relative of Heilongjiang soybean cultivars of globulin with tofu foods,Soybean Science,8(3):295-299.

Kapoor, A.C. and Y.P.Gupta, 1977, Changes in proteins and amino acids in developing soybean seed and effect of phosphorus nutrition, J. Sci. Food Agric., 28:113-120.

Wilson, C.M. et al., 1981, The extraction and seperation of barley glutelins and their relationship to other endosperm proteins, J. Exp. Bot., 32(131):1287-1293.

Zhang,X.B.,1992, A preliminary study on genetic variation of tofu processing characters of soybean landraces, Master thesis, Nanjing University Library.

Zhou,X.A., J.Y.Gai and Y.H.Ma,1992,Studies on the components of seed storage protein and their correlations in soybean,Soybean Science,11(3): 191-197.

Junyi Gai

Junpei Jin

Xiaobo Zhang

Table 1. ANOVA of total protein and components of storage protein

Trait	MSV	MSE	F
Total protein	12.85	1.61	7.997**
Globumin	13.63	2.37	5.743**
Albumin	0.96	0.08	11.864**
Prolamin	2.11	0.39	5.399**
Glutelin	8.81	0.71	12.457**

Table 2. The means and variability of total proein and components on storage protein.

Trait	$\bar{X}$	S	X_{min}-X_{max}	gcv (%)	h_2 (%)
Globulin %	31.11	2.61	23.10-38.80	7.63	70.34
Albumin %	2.55	0.69	1.30-5.00	25.96	84.45
Prolamin %	2.26	1.04	0.55-6.40	41.11	68.74
Glutelin %	5.88	2.10	1.90-13.35	34.22	85.14
Total protein (%)	46.27	2.54	38.5-52.5	5.13	77.77

Table 3. The correlations regarding the components of storage protein.

Trait	Albumin	Prolamin	Glutelin	Protein	Oil content	Dried tofu yield	Fresh tofu yield
Globulin	0.016	-0.042	-0.602**	0.383**	-0.143	0.285**	0.168*
Albumin		0.022	-0.313**	-0.014	0.380**	-0.277**	-0.191**
Prolamin			0.300**	0.011	-0.007	-0.125	-0.107
Glutelin				0.283**	0.282**	-0.068	0.019

Trait	Protein content in tofu	Oil content in tofu	Content of protein and oil in tofu	Amount of protein in tofu	Amount of oil in tofu	Amount of protein and oil in tofu
Globulin	-0.015	-0.356**	-0.131	0.079	-0.086	0.038
Albumin	-0.154	-0.055	-0.158	-0.161	-0.103	-0.164
Prolamin	09.145	-0.037	-0.144	-0.153	-0.106	-0.158
Glutelin	0.094	-0.081	0.059	0.041	-0.154	-0.015

Trait	Recovery of protein	Recovery of oil	Extract-abilitiy of protein	Coagul-ability of protein	Residue rate	Protein content in residue	Oil content in residue
Globulin	0.004	0.276*	-0.021	0.031	-0.034	0.104	-0.043
Albumin	-0.145	-0.070	0.223	-0.250	-0.026	0.153	-0.126
Prolamin	-0.163	-0.076	-0.027	-0.165	-0.133	0.027	-0.193
Glutelin	-0.008	-0.324*	-0.271	0.142	-0.077	-0.003	-0.174

Note: * and ** represent significant at 0.05 and 0.01 level, respectively.

Jilin Academy of Agricultural Sciences
Soybean Research Institute
Gongzhuling, Jilin Province 136100
The People's Republic of China

The study on the techniques of usage of wild soybean in soybean breeding*

Since 1979, 658 interspecific crosses were made to study the techniques of usage of wild soybean in soybean breeding. The objective of this study was to overcome viny growth habit and small size seed habit of progenies from interspecific hybrids, increasing hereditary diversification of cultivated soybean. Studying the methods and laws of usage of wild soybean in soybean breeding. Great progress has been gained in recent years. These results reveal :

1) Latent capacity of selection of major agronomic characters of progenies from different type crosses

Progenies from 20 interspecific crosses of G. max X G. soja and G. max X G. gracilis were used in this study. The results showed that in F_2 generation, except weight of 100 seeds, the rest of major agronomic characters from wild type crosses was higher than semi-wild type crosses. Except number of nodes of main stem, the genetic variation coefficient of major characters from wild type crosses were bigger than semi-wild crosses. Except weight of 100-seed, expected genetic advances of all other agronomic characters were bigger in all type crosses. In general, wild type crosses > semi-wild type crosses (Table 1). Plants with erect main stem (110cm in height), 350 pods per plant have been selected from determination crosses (female parentage is a cultivated soybean with determinate) and semi-determinate crosses (female parentage is a cultivated soybean with semi-determinate).

*This project is supported by NNSF of China

Table 1. The means, ranges, GCV's and expected genetic advance (GA) of major agronomic characters in F_2 generation

Characters	Cross Type	Ranges	Means	GCV.	GA
Plant height	Wild	145-330	204.0	24.04	55.3
(cm)	Semi-wild	100-335	168.4	23.37	50.8
No. nodes	Wild	16-35	23.4	24.48	3.1
on main	Semi-wild	10-32	18.5	25.74	2.7
stem					
No. pods	Wild	121-451	284.5	31.76	35.8
per plant	Semi-wild	91-345	226.1	27.44	26.4
No. seeds	Wild	263-1134	631.4	32.71	36.6
per plant	Semi-wild	207-936	503.2	23.38	22.1
Weight of	Wild	4.2-10.5	6.3	20.23	0.68
100 seeds	Semi-wild	7.0-13.6	9.5	16.50	0.62
(g)					
Protein	Wild	40.5-51.7	44.56	11.35	4.65
Content (%)	Semi-wild	39.6-49.4	43.16	9.71	3.87

2) Effect of parents on the techniques of parent selection

Progenies from 12 crosses and 8 backcrosses were used in this study. Three cultivated soybean with different influorescence were used as the female parents and two wild soybean (G. soja) and two semi-wild soybean (G. gracilis) were used as the male parents in these 12 according to NCII design. The results showed that it is feasible for overcoming the viny and seed habit if the cultivated genotype with determinate and semi-determinate influorescence, lower plant height, thick main stem and higher 100-seed weight was chosen as the male parentage in the interspecific cross. It seems that the choosing of wild parents is more important than that of cultivated soybean parents. It is more effective to select the semi-wild soybean as the parents in interspecific cross. The viny growth habit and small size seed habit can be overcome by only one backcross, if the parents are suitable.

3) The techniques of erect plant selection

The separate frequency of erect and semi-erect plant were significantly different in F_2 generation between different type interspecific crosses (Table 2). Except WH crosses (wild soybean with higher plant as the male), the erect and semi-erect plants for all other type crosses in generation were separated. The

separate frequence of erect and semi-erect plants from determinate, semi-determinate, SWL type crosses were higher than that of other type crosses. If the crosses were made with same cultivated parent and different wild parent, the separate frequence of erect and semi-erect plant were significantly different in these crosses. The stable erect type lines only separated from the erect and semi-erect plants in F_2 generation.

Table 2. The segregation frequency of erect and semi-erect plants, the mean of 100-seed weight in F_2 and F_4 generation

Cross Type	Range of segregation frequency of erect and semi-erect plants (%)		Weight of 100 seeds (g)	
	F_2	F_4	F_2	F_4
Det*	0-8.6	0-8.9	8.2	8.5
Semi-det	0-7.9	0-8.1	8.8	8.6
Int	0-0.4	0-1.0	9.3	9.3
WH	0	0	6.1	6.6
WL	0-2.5	0-2.8	7.4	7.2
SWH	0-5.0	0-6.8	9.3	9.4
SWL	0.4-8.6	1.0-8.9	9.6	9.8

* Det., Semi-det., Int. is cross of a cultivated soybean with determinate, semi-determinate and indeterminate influorescence as female parent

WH, WL is cross of a wild with higher and lower plant as male parent

SWH, SWL is a cross of a semi-wild higher and lower plant as male parent

4) The techniques of backcross

The increase of separation frequence of erect and semi-erect plant of progenies in F_1 generation when the backcross was randomly made. The raised weight of 100-seed in F_1 generation is small when the backcross is randomly made. If the genotype with erect and semi-erect higher 100-seed weight were selected to backcross in F_3 generation, the separate frequence of erect and semi-erect plant, weight of 100-seed of progenies from these backcrosses weresignificantly increased (Table 3). Some lines with cultivated type, higher yield and 100-seed weight over 20g were selected from progenies in these backcrosses.

Table 3. The segregation frequency of erect and semi-erect plant, the mean of 100-seed weight in F_2 of different backcross generation

Cross Type	Back Cross Generation	Frequency of erect and semi-erect plant (%)	100-seed weight (g)		
			$\overline{X}$	Range	CV (%)
Det X	F_1	6.72	11.7	5.1-18.1	25.33
Wild	F_3	34.74	15.9	7.8-20.3	23.75
Det X	F_1	10.11	14.1	6.7-19.3	21.28
Semi-wild	F_3	56.83	17.6	9.5-22.1	19.87
Semi-det	F_1	5.65	11.3	4.9-16.8	26.71
Wild	F_3	27.94	14.5	6.5-19.9	31.16
Semi-det X	F_1	8.95	14.3	7.3-20.3	18.94
Semi-wild	F_3	23.67	18.2	8.1-23.4	24.18

5) Selection techniques for agronomic characters of progenies from interspecific hybridization

On the basis of the erect plant were obtained: selection seed coat color, hilum color, seed coat luster and protein content. Then the choosing characters yield, when the erect plants obtained selection standard, for seed coat color, seed coat luster, etc. They should be released in early generation and the selection population should be larger. Last selection for these characters should be taken after F_6 generations. Viny growth habit and shattering characters should be eliminated from F_2 generation.

Some lines with over 350 pods per plant, 100-seed weight 10g and distance between nodes was shorter. Lines with over 150 pods per plant, 100-seed weight over 20g and erect plant have been obtained by using of the above techniques.

We have obtained 10 lines with protein content over 48%, four lines with protein content with over 50% coming from these lines.

The new soybean variety of small size seed "JilinXiao Li No. 1" was selected directly from the cross of G. soja and G. max. . The seed coat of this variety is yellow, hilum is white, seed is round, plant is erect, 100-seed weight is

9.5g, protein content is 44.89% and oil content is 16.14%. Now this variety has been exported to Japan. It is registered in Jilin province on April 1990.

Our results showed that it is possible to improve cultivated soybean by the use of wild soybean germplasm and the interspecific cross is a new way for soybean breeding.

References:

Wang, Jinling, et al. 1986. Effect of backcrossing on overcoming viny and lodging habit of cultivated X wild and cultivated X semi-wild cross. Soybean Sci. 3:181-188.

Yang, Guangyu, et al. 1993. Effect of G. max parents of different growth habit on performance of progenies in G. max X G. gracilis . Soybean Sci. 2:137-145.

Yang, Guangyu et al. 1993. A preliminary study on overcoming viny and small size seed habit of soybean interspecific hybrids. Soybean Sci. 4:276-282.

Yang Guangyu et al. 1990. A study on the use of G. soja to breed new soybean cultivars. Sci. Agri. Sinica 1:89-90.

Yang Guangyu
Zhen Huiyu
Han Chuen fen
Ji Feng
Hu Jinhai

NORTHEAST AGRICULTURAL UNIVERSITY
Department of Agronomy
Harbin, Heilongjiang 150030
P.R. China

Variations in seed protein content and 100-seed weight among different portions of soybean plants.

Soybean (*Glycine max* (L. Merrill) is famous for its high protein and high oil content in seeds and has become the major source of high protein feed supplements and of edible vegetable oils in the world. More and more research is being done in protein genetics and breeding. Therefore, precise and accurate analysis of protein in soybean seeds is very important. Sampling methods may affect the analysis greatly. Usually the best and largest seeds from a composite sample or a plant are used for the analysis of protein content. Are these seeds representative? The objectives of this experiment are (1) to study the variations of seed protein content and 100-seed weight among different portions of different types of soybean strains; (2) to ascertain the relationship between seed protein content and 100-seed weight.

In 1992, three indeterminate strains, 'Baojiao 85-5029', 'Dongnong 86-2-9' and 'Dongnong 42', three semi-determinate strains, 'Dongnong 87-138-5' , 'Dongnong 86-103-10' and 'Dongnong 90-532', two branch-type strains; 'Baoyou 12-1-17' and 'Harosoy,' were planted on May 8 in Xiangfang Farm, Harbin. The experimental design was a randomized complete block with two replications. One experimental plot contained four rows 5 m long, spaced 70 cm apart. The distance between plants in a row was 6 cm. Ten plants were harvested from the center rows of each plot. For indeterminate and semi-determinate strains, each plant was divided into three portions, top third, middle third and bottom third. For branch-type strains, each plant was divided into branches and main stem. Each portion was threshed separately for measurement of 100-seed weight and testing of seed protein content (on dry matter basis).

Results and Discussions: The results were shown in Table 1 and 2. For indeterminate soybeans, seed protein content of the top third of 'Dongnong 86-2-9' and 'Dongnong 42' was higher than the middle and bottom thirds, almost no difference between the middle and bottom thirds; seed protein content of 'Baojiao 85-5029' was relatively uniform from top to bottom of the plant. One hundred seed weight of the three indeterminate strains decreased from bottom to top except that

100-seed weight of the bottom third of 'Dongnong 42' was slightly smaller than the middle third. For semi-determinate soybeans, three strains have three different protein content variation patterns. Protein content of the middle third of 'Dongnong 87-138-5' was lower than the other two portions; protein content of the top third of 'Dongnong 86-103-10' was higher than the middle and bottom thirds; protein content of 'Dongnong 90-532' decreased from the bottom third to the top third. Seed size variation was similar to the indeterminate except for the relatively uniform seed size of 'Dongnong 87-138-3-5'.

The relationship between seed protein content and 100-seed weight for 'Dongnong 86-2-9', 'Dongnong 42', 'Dongnong 87-138-5' and 'Dongnong 86-103-10' were negative, but positive for 'Baojiao 85-5029' and 'Dongnong 90-532'.

For branch-type soybeans, protein content of main stem was higher than branches for both strains and 100-seed weight of the main stem was higher than branches for the two strains; seed protein content and 100-seed weight had a strong positive correlation.

The results indicate that both seed protein content and 100-seed weight have variations among different portions of a soybean plant. Therefore, for a single plant, it is very important to use all seeds of the plant or sample from every portion of the plant for the analysis of seed protein content. It is the best to use all seeds of a plant for the measurement of 100-seed weight of the plant. For a composite sample, we recommend using seeds with different sizes for the analysis of protein content. Choosing the best large seeds for protein content analysis is not a good recommendation.

Table 1. Protein content and 100-seed weight of different portions of a plant.

	Protein Content (%)			100-seed weight (g)		
	Top	Middle	Bottom	Top	Middle	Bottom
			Indeterminate			
Baojiao 85-5029	41.56	41.91	41.84	17.03	17.62	17.82
Dongnong 86-2-9	44.01	41.51	41.48	14.32	15.61	16.03
Dongnong 42	46.63	45.80	45.95	21.23	23.79	23.55
			Semi-determinate			
Dongnong 87-138-5	41.76	40.85	41.71	17.63	17.96	17.27
Dongnong 86-103-10	42.56	40.38	40.84	16.23	17.83	18.31
Dongnong 90-532	37.40	39.84	40.48	13.77	14.91	16.28

Table 2. Protein content and 100-seed weight of main stem and branches of a plant.

	Protein content (%)		100-seed weight (g)	
	Main stem	Branches	Main stem	Branches
Baoyou 12-1-17	45.85	45.25	16.31	15.74
Harosoy	45.34	43.92	16.27	15.08

References:

Collins, F.I. and J.L Cartter. 1956. Variability in chemical composition of seed from different portions of the soybean plant. Agronomy Journal, 48:216-219.

Escalante, Eduardo E. and J. R. Wilcox. 1993. Variation in seed protein among nodes of normal- and high-protein soybean genotypes. Crop Sci. 33(6):1164-1166.

Escalante, Eduardo E. and J. R. Wilcox. 1993. Variation in seed protein among nodes of determinate and inteterminate soybean near-isolines. Crop Sci 33(6):1166-1168.

Zhang Guodong

NORTHEAST AGRICULTURAL COLLEGE
Harbin, Heilongjiang 150030
Peoples Republic of China

The evaluation and utilization of genetic potential of semicultivated soybeans

Abstract: Genetic potential, breeding effectiveness of progenies from crosses between semicultivated and cultivated types of soybean were studied. The results revealed that progenies from crosses between cultivated and semicultivated soybean, had higher plant height, more luxuriant growth, more seeds and pods per plant, lower seed-stem ratio and less 100-seeds weight than those of intervarietal crosses. Different types of soybean had significantly different combining ability on main agronomic characters. Semicultivated soybean parents had promoting effect on plant height, number of nodes, number of branches, number of pods and seeds to their progenies, while semideterminate soybeans had reducing effect on plant height, length of internodes, and promoting effect on 100 seeds weight, and seed-stem ratio. Crosses including semicultivated germplasm had greatest genetic potential. The infusing of semicultivated germplasm to the cultivated soybean could increase number of seeds and pods per plant significantly, and consequently could enhance selecting potential on yield. If parents of semicultivated x cultivated hybrids were handled suitably, plant height, 100-seeds weight of the progenies could restore to the cultivated form in F_2 generation. The crosses including semicultivated soybean germplasm could also be used to select minute-seeded and other specific new varieties.

Introduction: Soybean is one of the world's most important crops. For a crop of such international significance, it's future of breeding still depends upon a relatively narrow base of genetic resources. In such a situation, the wild soybeans were of great interest as a potential genetic resource (Hadley and Hymowitz, 1973; Brown and Grant, 1985). The main purpose of using wild soybean
is to transfer high protein, high yield genes from wild to new cultivars and to abandon those undesired characters of the wild soybean such as viny stem, black seed coat color, and small seeds (Carper and Fehr, 1980; Wang Jin Ling, 1986). Gai et al, 1982, indicated that two or three cycles of backcross were enough to diminish the wild characters. In contrast to the use of wild soybean, the use of semicultivated soybean as parent for crossing was relatively neglected.

Semicultivated soybean has some desired characteristics including tolerance to adverse environments, pest and disease resistance, and multi-seed characters. The potential of introducing semicultivated soybean to improve cultivated soybeans has

rarely been reported. As soybean originated in China, semicultivated soybean germplasms are very rich, so we have the advantage to study and to exploit the genetic resource of this close relative of cultivated soybean - semicultivated soybean.

Materials and Methods: Fifteen crosses were made among semicultivated, formerly released indeterminate and currently released semideterminate soybean cultivars by diallel crossing method. Semicultivated type of soybeans are intermediate between wild and cultivated soybeans in evolutionary rank, with coat colored seeds, more braches, luxuriant and more or less viny growth. All parents, F_1 and F_2 progenies were grown in the field of NEAC Experimental station in Harbin, 1990, by randomized block design with three replications. The six parents are shown in Table 1.

Table 1. Performance of cultivars or lines of different types to soybean used as crossing parents

Variety	Type	Plant height	100-seeds weight	Seed-stem ratio	Seed coat color
Long79-6804	Semicultivated	87.00	14.80	0.7014	Brown
Lumeshido	Semicultivated	74.19	8.32	0.7752	Green
Jinshanpu	Cultivated (Indeterminate)	88.00	15.96	0.9005	Yellow
Manchang Jin	Cultivated (Indeterminate)	80.00	17.17	0.8795	Yellow
Hefeng 25	Cultivated (semideterminate)	49.66	16.51	1.1052	Yellow
Dongnong 72-806	Cultivated (semideterminate)	54.09	13.22	0.9862	Yellow

Results and Discussion:

The performance and distribution of main agronomic characters of progenies for different type of crosses

The performance of plant height in the F_1 and F_2 for 4 of the 15 crosses could be seen in Table 2. The order of plant height from lower to higher was semideterminate x semideterminate < semideterminate x indeterminate < semideterminate x semicultivated < indeterminate x semicultivated. The mean value of plant height of semideterminate x indeterminate crosses was similar to that of the semideterminate x semicultivated crosses. In addition, these two types of crosses performed significantly lower plant height than that of indeterminate x semicultivated cross. Mode of genetic segregation

segregation showed that in the semideterminate x semideterminate cross, 80% progenies were below 70 cm in height, in the semideterminate x indeterminate and semideterminate x semicultivated crosses, the height of progenies below 80 cm were 80% and 70%, respectively, while in the indeterminate x semicultivated cross, 90% plants were above 90 cm. Therefore, it seems that by use of semicultivated soybean, cultivars as parents to cross with semicultivated soybean germplasm, the progenies can restore to the cultivated form in plant height in F_2 generation without being backcrossed.

Table 2. Agronomic performance in plant height for 4 of the 15 crosses

		F_1	F_2			
Code	Cross (Type)	$\overline{X}$	$\overline{X}\pm S$	CV	Segregation	Deviation of extreme value
89-1	Hefeng 25 x Dongnong 72-806 (Semideterminate x Semideterminate)	58.73	63.6±8.23	10.26	80% below 70 cm	36
89-4	Hefeng25 x Manchangjin (Semideterminate x Indeterminate	81.60	72.29±11.72	12.66	80% below 80 cm	45
89-6	Hefeng25 x Lumeshido (Semideterminate x Semicultivated)	83.53	76.08±12.28	15.67	70% below 80 cm	55
89-15	Mangchangjin x Long79-6804 (Indeterminate x Semicultivated)	+95.80	87.04±12.65	14.54	90% above 90 cm	64

Difference in coefficient of variability among crosses is significant. In the crosses of 89-1 and 89-6, where Hefeng 25 was the common parent, C.V. in the cross 89-1 was higher than that of 89-6. The same results revealed in 89-4 and 89-6 crosses, where C.V. in the 89-6 was higher than that of 89-4. These results suggest that the infusing of semicultivated soybean germplasm to the cultivated soybean can increase the selecting potential in soybean cross breeding.

The performance of seed-stem ratio of different type of soybeans can be seen in Table 1. The order of seed-stem ratio from lower to higher is: semicultivated soybean < indeterminate soybean < semideterminate soybean. Obviously, the higher the evolutionary rank of soybean is, the higher the seed-stem is. Highly significant correlation coefficients (r) between the parental mean performance of seed-stem ratio and that of progeny population (F1=0.8261**, F2=0.8659**n=15) demonstrate that the higher the parents in seed-stem ratio, the higher the ratio of the progenies will be. In

order to obtain lower seed-stem ratio offspring in soybean breeding program, semicultivated soybean germplasm should be used as parents. The highly significant correlation coefficients between parent means and means of F_1 and F_2 population in 100-seeds weight are 0.8568 and 0.9018, respectively. Therefore, 100-seeds weight of soybean parents influence greatly that of their offspring. From Table 3, it can be seen that the crosses of 89-14 and 89-15 (cultivated X semicultivated) took Manchangjin (cultivated) as common parent which had mean 100-seeds weight of 17.17g, however, the 100-seeds weight of the semicultivated parents were different. The F_2 progenies of the cross 89-14 had the mean 100-seeds weight of 12.69 and more than 60% segregates were 11-13g in 100-seeds weight. This is due to the fact that the male semicultivated parent, Lumeshido was 8.32g in 100-seeds weight. In the cross 89-15, the 100-seeds weight of male semicultivated parent Long79-6804 was 14.8g. Consequently, the mean value of F_2 was 16.3g and more than 63% segregates were more than 15.0g in 100-seeds weight. It seems that, for the purpose of obtaining more segregants with 100-seeds weight restoring to that of the cultivated form in F_2 generation, the higher 100-seeds weight semicultivated soybean germplasm should be used as parent and this may give a better opportunity for selecting larger seeded offspring without being backcrossed with cultivated soybean. In breeding of minute seed soybean, the semicultivated soybean germplasm would donate minute seed genes. This point can be demonstrated from 89-12 and 89-13 crosses, too.

Table 3. Agronomic performance F_2 on 100-seeds weight for 6 of the 15 crosses

Code	Crosses (Type)	The mean value ♀	♂	M	F_1	F_2
89-2	Jinshanpu x ManchangJin (Cultivated x Cultivated	15.96	17.17	16.57	16.48	16.82
89-3	Long79-6804 x Lumeshido (Semicultivated x Semicultivated)	14.80	8.32	11.56	12.97	11.80
89-12	Jinshanpu x Lumeshido (Cultivated x Semicultivated)	15.96	8.32	12.14	13.47	11.71
89-14	MangchangJin x Lumeshido (Cultivated x Semicultivated)	17.17	8.32	12.75	14.92	12.60
89-15	MangChangJin x Long79-6804 (Cultivated x Semicultivated)	17.17	14.80	15.38	17.17	16.33
89-13	Jinshanpu x Long79-6804 (Cultivated x Semicultivated)	15.96	14.80	15.99	17.43	15.35

Code	Crosses (Type)	The distribution of 100-seeds weight (%) 11-13	13.1-15	15.1-18	18.1-20	720
89-2	Jinshanpu X ManchangJin (Cultivated x Cultivated)	0	12.22	61.77	19.01	7.01
89-3	Long79-6804 x Lumeshido (Semicultivated x Semicultivated)	81.11	15.56	3.34	0.00	0.00
89-12	Jinshanpu x Lumeshido (Cultivated x Semicultivated)	80.00	16.67	3.33	0.00	0.00
89-14	MangchangJin x Lumeshido (Cultivated x Semicultivated)	60.00	27.78	11.11	1.11	0.00
89-15	MangChangJin x Long79-6804 (Cultivated x Semicultivated)	10.01	36.67	44.44	8.88	0.00
89-13	Jinshanpu x Long79-6804 (Cultivated x Semicultivated)	3.24	26.66	55.56	14.44	0.00

Comparison of general combining ability among different types of soybean germplasm

General combining ability of different types of soybean parents can be seen in Table 4. Different types of soybean had significantly different general combining ability on the agronomic characters. Semicultivated soybean parents had the largest positive general combining ability on plant height, branch number, length of internodes, pods and seeds number per plant The general combining ability on seed-stem ratio is highly negative. In contrast, semideterminate cultivated soybean had the contrary tendency, and indeterminate cultivated soybean was intermediate between those of semicultivated and semideterminate cultivated soybeans. It seems that semicultivated soybean may be the type donor for high yield and luxurious growth gene, while the semideterminate cultivated soybean may be the type as gene donor to reduce the primitive performance.

Table 4. The general combining ability of main agronomic characters of the parents

Cultivar (type)	Plant height	Branch number	Length of internodes	Seed-stem ratio	Pods per plant	Seeds per plant	Hundred seed weight	Yield per plant
Hefeng25 (Semideterminate)	-9.22	-1.33	-0.49	0.38	-15.40	-33.78	1.43	-2.45
Dongnong72-806 (Semideterminate)	-9.18	-1.28	-0.23	0.30	-8.64	-9.56	0.08	-1.54
Manchangjin (Indeterminate)	0.24	0.29	0.44	0.10	2.57	5.02	0.24	4.04
Jinshanpu (Indeterminate)	2.24	0.92	-0.09	-0.07	-1.94	1.09	0.87	2.80
Long79-6804 (Semicultivated)	11.47	1.32	0.24	-0.64	12.98	14.70	0.49	1.23
LuMeshido (Semicultivated)	4.44	1.07	0.13	-0.12	10.43	22.56	-3.11	-4.70

Comparison of genetic potential between the crosses with and without semicultivated soybean germplasm

From Table 5, it can be seen that the crosses with semicultivated soybean germplasm performed higher plant height, higher branch and node number, and more luxuriant growth, lower seed-stem ratio. In addition, progenies of such crosses had more seeds and pods per plant and less 100-seeds weight than those of intervarietal crosses.

Table 5. The mean value and coefficient of variation of main agronomic characters in different type of crosses

Type of crosses	Parameter	Plant height	Node number	Branch number	Internode length	Seed-stem ratio	Yield per plant	Pods per plant	Seeds per plant	100-seeds weight
Crosses with semicultivated soybean germplasm	$\overline{X}_{F1}$	90.49	19.78	4.33	4.67	1.38	33.37	109.85	223.84	14.75
	$\overline{X}_{F2}$	82.56	18.27	4.00	4.54	1.21	29.26	100.08	215.54	13.83
	CV	14.30	17.49	55.38	20.73	32.44	49.79	44.96	58.32	15.76
Crosses without semicultivated soybean germplasm	$\overline{X}_{F1}$	75.36	17.57	2.81	4.28	1.89	29.61	73.16	181.64	15.76
	$\overline{X}_{F2}$	74.86	17.79	2.89	4.33	1.81	31.30	70.14	195.63	15.96
	CV	13.09	13.82	45.87	15.87	25.99	43.90	33.79	42.68	13.37

Difference in coefficient variability (C.V.) of main agronomic characters between crosses with and without semicultivated soybean as parent is significant. Crosses with semicultivated soybean germplasm had higher C.V. on the agronomic characters than that of intervarietal crosses. Therefore, the selection potential of the main agronomic characters is higher in crosses with semicultivated soybean germplasm as parent than those without semicultivated soybean germplasm.

The coefficient of variability (CV) broad sence heritability (H_B) and expected genetic advance (G_A) on yield characters was also different among crosses (Table 6). The crosses between different types of soybean had higher C.V., H_B, and G_A than those of crosses within the same type of soybean. It is suggested that the infusing of semicultivated soybean germplasm to the cultivated soybean can increase number of seeds per plant and number of pods per plant significantly and this enhance the selection potential on yield.

Table 6. The genetic parameter for the main yield characters in different type of crosses

Type of crosses	Parameter	Yield per plant	Seeds per plant	Pods per plant
Semideterminate x Semideterminate (Cultivated x Cultivated)	$\overline{X}$	20.91	132.88	50.58
	CV	37.29	35.11	32.16
	H_B	10.34	8.64	10.23
	G_A	5.17	28.25	10.75
Semideterminate x Indeterminate (Cultivated x Cultivated)	$\overline{X}$	30.11	195.01	75.71
	CV	46.12	45.15	42.81
	H_B	50.52	70.14	42.12
	G_A	20.33	151.9	52.99
Semideterminate x Semicultivated (Cultivated x Semicultivated)	$\overline{X}$	31.04	214.15	100.09
	CV	47.84	48.18	43.15
	H_B	74.73	74.94	57.86
	G_A	26.44	183.99	67.67
Indeterminate x Semicultivated (Cultivated x Semicultivated)	$\overline{X}$	28.71	217.12	95.14
	CV	52.11	65.78	46.73
	H_B	59.73	72.30	54.97
	G_A	23.84	238.62	67.90

Conclusion: The semicultivated soybean germplasm is a very useful resource to broaden the genetic constitution of soybean. Based on the results presented, we believe that to transfer the genotypic constituents of semicultivated to the cultivated soybean we could increase pod and seed number of the progenies effectively and also cause an increase in genetic potential of the main agronomic characters. The key points of introducing semicultivated in cross breeding are primarily collecting semicultivated soybean germplasm with higher 100-seeds weight (lower 100-seeds weight materials hould be used for breeding of minute seed soybean variety), to cross with semideterminate cultivars with stiff stem. It is suggested that the combination of these two genetic donor (semideterminate x semicultivated) might give the best result in producing high-yield and good performing offsprings. With crosses of such combination, selection for good agronomic performing offspring might properly begin in F_2 generation, and need not being backcrossed with cultivated soybeans. The breeding procedure is more simple and breeding duration is shorter than that of using wild soybean as crossing parent.

References:

Ertl, D.S. et al. 1985. Agronomic performance of soybean genotypes from Glycine Max X Glycine Soja crosses. Crop Sci. 25:589-592.

Hartwig, Edgar E. et al.. 1972. Utilization of soybean germplasm traits in soybean improvement program. Crop Sci. 12:856-859.

Schoener, C.S. and Fehr, W.R. 1979. Utilization of plant introduction in soybean breeding populations. Crop Sci. 19:185-188.

Thorne, J.C. W.R. Fehr. 1970. Exotic germplasm for yield improvement in 2 way and 3 way soybean crosses. Crop Sci 10:577-578.

Thorne, J.C. W.R. Fehr. 1970. Incorporation of high protein, exotic germplasm in soybean opulations by 2-way and 3-way crosses. Crop Sci 10:652-655.

Vello, N.A. et al., 1984. Genetic variability and agronomic performance of soybean population developed from plant introductions. Crop Sci. 24:511-514.

WangJinLing, et al. 1986. Correlation and path analysis between protein and the other quantitative traits in G. soja and G. gracilis species. J.Northeast Agric Coll. No 4:1-3.

WangJinLing et al. 1986. The effect of backcross on overcoming viny trait of the hybrid between G. max and G. soja . Soybean Sci. 5.3:182-185.

Yang Qi

Wang Jin Ling

NORTHEAST AGRICULTURAL COLLEGE
Harbin 150030
The People's Republic of China

Study on the performance of different types of soybean under different fertility level conditions

Abstracts: Six different types of soybean and their progenies were evaluated under three different fertility level conditions. The results suggested that different types of soybean and their progenies performed different adaptabilities. Soybean with higher plant height, more luxuriant growth and lower evolutionary level adapted to impoverished soil. Soybean with well-developed main stem, greater 100-seeds weight and higher evolutionary level adapted to fertile soil. Soybean with suitable plant height, and branching capability, higher seed-stem ratio, adapted to general soil conditions. The ecological yield had significantly positive correlation with biomass yield for all types of soybean under different ecological conditions. Based on the results of this study, a shifting ecological breeding concept was recommended.

Introduction: The importance of biotype and its use in soybean breeding procedure and production has not been fully realized, since major emphasis has been chiefly placed on the genetic and selecting strategies in soybean. Research conducted to determine soybean performance under different ecological conditions could provide useful leads for better breeding of new soybean varieties. The major objective of this study was to identify and estimate the effect of ecological conditions, especially the fertility level that influences the performance of soybean varieties. The study also attempts to discern whether different types of soybean variety adapted to different ecological conditions.

Materials and Methods: Crosses were made among semicultivated, formally released indeterminate and currently released semideterminate soybean cultivars. Each portion of all parents and their F_2 progenies were grown in the field of Lanxi County Agricultural Research Institute. NEAC Experimental station, Shueihua Agricultural Research Institute, representing Heilongjiang alkali-saline soil, general soil and fertility soil conditions, respectively (Table 1), by randomized block design with three replications.

Table 1. Soil fertility index of three locations

Fertility index	Lanxi	Harbin	Shueihua
Complete nitrogen(%)	0.0745	0.0768	0.0959
Alkali hydrolysis nitrogen (ppm)	6.9460	115.7000	173.6600
Complete phosphate (%)	0.0133	0.0255	0.0760
Available phosphate(%)	13.0000	28.0000	67.0000
Quick effective potassium (ppm)	83.0000	180.0000	200.3000
Organic matter (%)	1.47	1.53	2.10

Results and Discussion:

Analysis of stability of yield for different types of soybean and their F_2 generation

Variance analysis suggested that there are significant differences in soybean seed yield among varieties and among locations at 0.05 and 0.01 level, respectively (F=2.54* F=8.69**). Moreover, the interaction of variety x location was significant at 0.05 level. The results indicate that there are significant differences in seed yield among differenty types of soybean variety and this was significantly influenced by the ecological conditions. From Table 2 it can be seen that Dongnong72-806 had the lowest mean value and highest S^2_D and C.V. in seed yield per plant of all varieties. These results indicate that variety with narrow adaptability and poor stability in yield might easily be influenced by the environment. Hefeng 25 had the lowest Sd^2 and C.V., but had higher mean value in yield. It seems that the highest stability and adaptability was the main reason that this variety rapidly spread in large areas. ManChangjin and JinShanpu having higher mean value, lower S^2_D and C.V. in seed yield per plant suggested that the higher stability and adaptability might be the main reason that the two varieties had contributed more than 60% genetic source of Heilongjiang soybean varieties.

Table 2. Parameter of stability of yield per plant for six varieties

Varieties	Mean Value X	Mean Square Deviation S^2_D	Variation Coefficient C.V.
Hefeng 25	21.60	0.0131	2.03
Dongnong72-806	18.69	62.46	28.54
Manchangjin	19.21	1.07	2.50
Jinshnpu	24.86	11.03	5.65
Lumeshido	12.85	31.53	22.36
Long79-6804	18.70	10.01	19.42

The variance anlysis indicates that the interaction of location x cross was significant at 0.01 level (F=5.41**) in seed yield. This means that crosses performed differently under different ecological conditions. From Table 3 it can be seen that cross Hefeng 25 x Dongnong 72-806 (semideterminate x semideterminate) had the lowest mean value at Lanxi and the highest at Shueiha in seed yield. On the contrary, cross Jinshanpu x Lumeshido (indeterminate x semicultivated) had the highest mean value at Lanxi and lowest mean value at Shueihua in seed yield. Hefeng25 x Jinshanpu (semideterminate x indeterminate) had the highest mean value in seed yield at Harbin. It seems that different types of soybean varieties and their progenies performed differently in seed yield at different fertility level conditions. On the results presented, we concluded tht soybean with higher plant height, more luxuriant growth and lower evolutionary level adapted to the impoverished soil conditions, such as Lanxi location. In contrast, soybean with well-developed main stem, greater 100-seeds weight and higher evolutionary level, adapted to the fertile soil conditions such as Shueihua location.

Table 3. Mean yield per plant from three locations for different crosses in F_2 generation

Crosses	Lanxi	Harbin	Shueihua	Mean
Hefeng25 x Dongnong72-806	20.84	23.39	29.75	24.66
Hefeng25 x Jinshanpu	30.23	38.10	27.64	31.99
Dongnog72-806 x Lumeshido	22.94	27.99	24.70	25.21
Jinshanpu x Lumeshido	29.47	27.17	21.64	25.09

Correlation of seed yield with main agronomic characters under different ecological conditions

The ecological condition could have significantly influenced the correlation of seed yield with agronomic characters (Table 4). Under the impoverished soil condition of Lanxi, seed yield had significant postive correlation with nodes and branch number, pods and seed number and had significant negative correlation with 100-seeds weight. Consequently, soybean with higher plant height, more luxuriant growth and lower evolutionary level adapted to the impoverished soil conditions. Subsequently, selecting varieties with such characters could increase the yield potential in the lower fertility soil conditions.

Table 4. The correlation coefficient of the yield per plant with the main agronomic characters in different locations

Characters	Lanxi		Harbin		Shueihua	
	Varieties	Crosses	Varieties	Crosses	Varieties	Crosses
Plant height	0.3151	0.4367	0.2230	0.3994	-0.9132**	-0.8618
Nodes per plant	0.6740**	0.8062**	0.6718**	0.8034**	-0.5474**	-0.4958*
Number of branches	0.6556**	0.7863**	0.6324**	0.4720	-0.6434**	-0.8469
Pods on main stem	-0.1094	-0.0941	0.2070	0.3735	0.6458**	0.9298**
Pods on branches	0.6499**	0.7285**	0.4735	0.6232**	-0.3104	-0.5902**
Pods per plant	0.3864	0.6043**	0.6303**	0.6376**	0.0478	-0.0649
Seed-stem ratio	-0.3569	`-0.3438	0.3175	0.3032	0.8017**	0.7187**
Seeds per plant	0.6981**	0.8022**	0.8428*	0.6577**	0.9248**	-0.0978
100-seeds weight	-0.5615*	-0.5437*	0.7467**	0.5835**	0.9201**	0.5973
Harvest index	-0.3046	-0,3253	0.2625	0.2069	0.7771*	0.6464**

On the contrary, it can be seen from figure 1, in the fertile soil condition of Shueihua, seed yield had significant positive correlation with 100-seeds weight, seed-stem ratio and pods on the main stem and had significant negative correlation with plant height, nodes and branch number, etc/ Eight of ten analyzed correlation coefficients in Shueihua had opposite direction from that of Lanxi. As a result, soybean with well-developed main stem, greater 100-seeds weight and higher evolutionary level adapted to fertile soil. Thus selected varieties with such characters could increase the yield potential of this area.

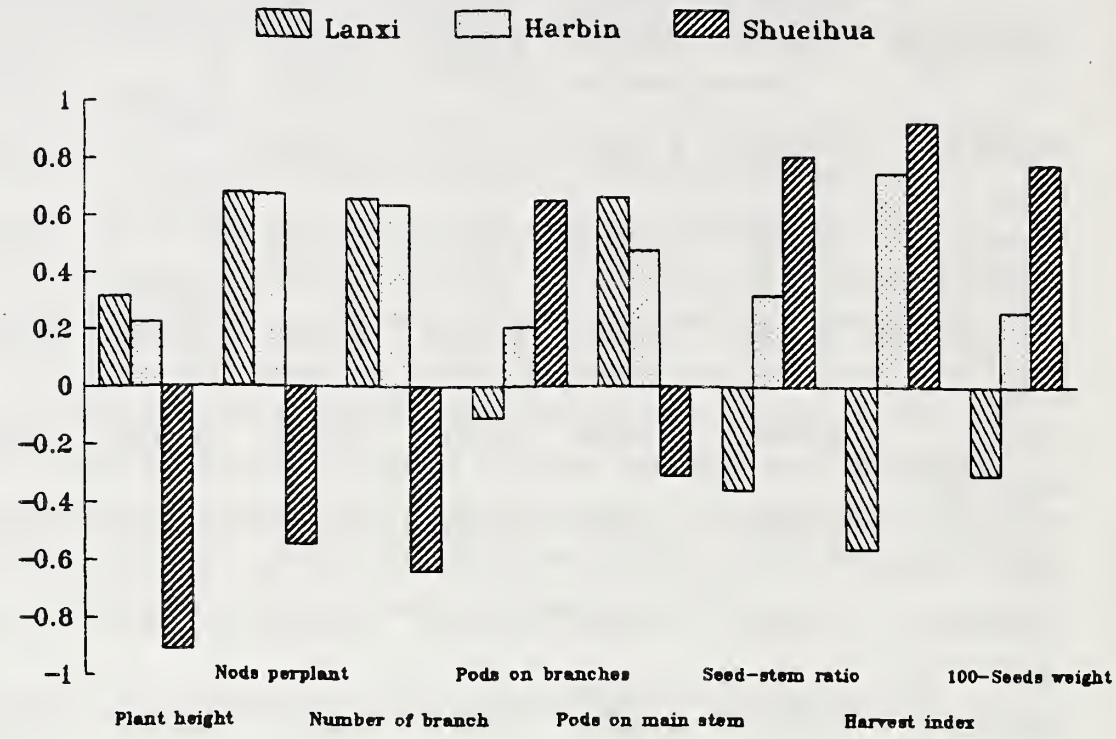


Figure 1. The correlation coefficient of the yield per plant with the main agronomic characters under different locations

Under the general soil conditions of Harbin, the correlation coefficients of seed yield with plant height, nodes number, branch number and pods number were in the same trend with those of Lanxi. Whereas, the correlation coefficients of seed yield with 100-seed weight, harvest index, seed-stem ratio were in the same trend with those in Shueihua (Figure 1). Obviously, since the fertility level of Harbin is in the middle of Lanxi and Shueihua, the correlation coefficients of yield with morphological characters in Harbin are also between those in Lanxi and in Shueihua. It seems that soybean with suitable plant height, suitable branching capability and higher seed-stem ratio, adapted to the general soil conditions.

The correlation analysis of economical yield with biological yield on the different fertility level conditions

It can be seen from Table 5 that seed yield had significant positive correlation with the biomass yield at all locations. Those results suggested that for any types of soybean varieties and at any type of fertility level soil conditions, the ecnomical yield had closer relationship with biomass yield. As a result, in the soybean selecting strategies, the biomass yield should be taken into account as an important selecting character.

Table 5. The correlation coefficient of the yield per plant with the seed-stem ratio and other characters

Location	Weight per plant at harvest	Harvest index	Seed-stem ratio	Weight of stem and branches
Lanxi	0.9848**	0.1598	-0.2435	0.7267**
Harbin	0.9415**	0.4055	0.0424	0.8341**
Shueihua	0.8203**	0.7455**	0.7002**	0.2126

The correlation of seed yield with harvest index is greatly influenced by the environmental conditions. Under the fertile soil conditions of Shueihua, the seed yield had significant positive correlation with harvest index and seed-stem ratio at 0.01 level. Under the general and impoverished soil conditions, the seed yield had nonsignificant correlation with those characters. This has meant that in the more fertile soil conditions, we should give special attention to increase the seed-stem ratio in order to use the dry matter transforming potential so as to increase the yield potential. In the general and impoverished conditions, we should pay more attention to select the variety with higher biomass yield and the relationship of the biomass yield with seed yield.

Conclusion: Ecotype play an important role in soybean breeding procedure. This study provides strong support for the view of different types of soybean adapted to different ecological conditions. Consequently, the ecological conditions determine more or less the soyean breeding objective of that region. In the higher fertility soil conditions, we should pay more attention not only to increase biomass yield, but also to increase the seed-stem ratio, as a result, to increase the yield. Under the impoverished conditions, we should give special attention to increased biomass yield in order to increase seed yield on the basis of these

results a shifting ecological breeding concept is recommended. This concept including 1)Establishing higher fertility soil and general fertility soil conditions in selecting nursery separately 2) Selecting F_2 and F_3 progenies under higher and general soil fertility levels separately 3) Sending lines to their adaptive regions to test yield performance and stability 4)Releasing the new adapted varieties to their adaptive regions.

References:

Bearer, J.S. et al. 1981. Yield stability of determinate and indeterminate soybean adapted to the northern United States. Crop Sci. 21:449-454.

Chang, J.E. 1982. Yield and agronomic performance of semideterminate and indeterminate soybean stem types. Crop Sci. 22:97-101.

Foley, T.C. et al. 1986. Performance of related determinate and indeterminate soybean lines. Crop Sci. 26:5-8.

Garland, M.L. et al. 1981. Selection for agronomic characters in hill and row plots of soybeans. Crop Sci. 21:591-595.

Hurtung, R.C. 1981. Modification of soybean plant architecture by genes for stem growth and maturity. Crop Sci. 21:51-56.

Meng, Qingxi et al. 1984. The study of selecting effect of soybean progenies on different ecological conditions. Soybean Sci. (China) 2:255-264.

Wang Jinling. 1981. Ecological characters and variety resource of soybean. China Oil Crop. 1:1-9.

Yang Qi

Wang Jinling

NORTHEAST AGRICULTURAL COLLEGE
Harbin, Heilongjiang 150030
People's Republic of China

The combining ability analysis for three different types of soybean and their progenies

Abstract: 15 crosses were made among and within three obviously different types of soybean by diallel crosses. The results suggested that different types of soybean had significantly different general combining abilities on the main agronomic characters. Semicultivated soybean parents had promoting effect on plant height, length of inter-nodes, number of branches, number of pods per plant, seed number per plant and had reducing effect on the seed-stem ratio and harvest index. Semideterminate soybean had reducing effect on plant height, length of inter-nodes, and promoting effect on seed-stem ratio. It was observed that different types of crosses had significantly different breeding value and total combining abilities on the morphological characters. As the two parents of a cross evolving from semicultivated to cultivated type, the mean value, breeding value and total combining abilities of seed-stem ratio, harvest index and other characters of the progenies had simultaneous increasing tendency, but those of plant height, number of branches, length of inter-nodes and other characters had decreasing tendency.

Introduction: Knowledge of the soybean combining ability in agronomic characters can provide important information for effective utilization of soybean germplasm. Previous researchers were more attentive to soybean combining ability of the different agronomic characters to determine the mode of gene action (Chenghenghe, 1982, Ohm, 1973, Kunta, 1985). Additionally, considerable research has dealt with the soybean combining ability of different geographically originating cultivars (Gai Junyi, 1983, Kaw, 1981, Paschal, 1985). The combining ability of different types of soybean cultivars is not fully understood. In this paper, we want to elucidate the difference of combining ability in agronomic characters for different types of soybean in order to utilize different types of soybean germplasm more effectively in soybean breeding.

Materials and Methods: 15 crosses were made among semicultivated, formerly released indeterminate and currently released semideterminate soybean cultivars by diallel crossing method. Semicultivated type of soybeans are intermediate between wild and cultivated soybean in evolutionary rank, with colored seeds coat, more branches, luxuriant and more or less viny growth. All parents, F_1 progenies were grown in the field of NEAC Experimental station in Harbin, 1990 by randomized block

design with three replications. The parental combinations are shown in Table 1. Statistical analysis method is shown in Table 2 and Table 3.

Table 1. The parentage combination and type of crosses

Code	Cross	Type
1	Hefeng25 × Dongnong72–806	Semideterminate × Semideterminate
2	Jinshanpu × Mangchangjin	Indeterminate × Indeterminate
3	Long79–6804 × Lumeshido	Semicultivated × Semicultivated
4	Hefeng25 × Mang Changjin	Semideterminate × Indeterminate
5	Hefeng25 × Jinshanpu	Semideterminate × Indeterminate
6	Dongnong72–806 × Manchangjin	Semideterminate × Indeterminate
7	Dongnong72–806 × Jinshanpu	Semideterminate × Indeterminate
8	Hefeng25 × Long79–6804	Semideterminate × Semicultivated
9	Dongnong72–806 × Long79–6804	Semideterminate × Semicultivated
10	Hefeng25 × Lumeshido	Semideterminate × Semicultivated
11	Dongnong72–806 × Lumeshido	Semideterminate × Semicultivated
12	Manchangjin × Long79–6804	Indeterminate × Semicultivated
13	Manchangjin × Lumeshido	Indeterminate × Semicultivated
14	Jinshanpu × Lumeshido	Indeterminate × Semicultivated
15	Jinshanpu × Long79–7804	Indeterminate × Semicultivated

Table 2 The analysis of combining ability by griffing 4

Source of variation	DF	SS	MS	Immobile model	Random model
General combining ability	P−1	S_g	Mg	$\sigma^2 + (P-2)\frac{1}{P-1}\sum_i g_i^2$	$\sigma^2 + \sigma_s^2 + (P-2)\sigma_g^2$
Specific combining ability	$\frac{1}{2}P(P-3)$	S_s	Ms	$\sigma^2 + \frac{2}{P(P-3)}\sum_{i<j}\sum S_{ij}^2$	$\sigma^2 + \sigma_s^2$
Error		S_e	Me	σ^2	σ^2

Table 3 Estimation of effect

Group mean $\hat{\mu}$	$\frac{2}{P(P-1)} X_{..}$
General combining ability $\hat{g_i}$	$\frac{1}{P(P-2)}(PX_{i.} - 2X_{..})$
Specific comining ability $\hat{s_{ij}}$	$X_{ij} - \frac{1}{(P-2)}(X_{i.} - X_{j.}) + \frac{2}{(P-1)(P-2)} X_{..}$

Breeding value = Tatal general combining ability of two parents

Tatal combining ability = General combining ability+specific combining ability

Results and Discussion: The comparison of general combining ability (gca) for different types of soybean in main agronomic characters:

From Table 4 it can be seen that the semicultivated soybean, Long 79-6804 and Lumbeshido, have the highest positive and semideterminate soybean. Hefeng 25 and Dongnong 72-806 have the lowest negative gca in seeds and pods number per plant, respectively. Those results indicate that the semicultivated soybean have promoting effect on yield potential, especially the seeds and pods number per plant.

Mangchangjin have higher positive gca on seeds and pods number per plant and the highest positive gca in seed yield per plant. On the basis of this result, we conclude that the highest gca on seed yield and higher gca on pods and seeds number per plant might be the main reason why this cultivar has contributed more than 60% of genetic source to cultivars grown in Heilongjiang.

Table 4. The general combining ability of main agronomic characters of the parents

Cultivar (type)	Plant height	Branch number	Length of inter-nodes	Seed-stem ratio	Pods per plant	Seeds per plant	Hundred Seed Weight	Yield per plant
Hefeng 25 (semideterminate)	-9.22	-1.33	-0.49	0.38	-15.4	-33.78	1.43	-2.45
Dongnong72-806 (semideterminate)	-9.18	-1.28	-0.23	0.30	-8.64	-9.56	0.08	-1.54
Manchangjin (Indeterminate)	0.24	0.29	0.44	0.10	2.57	5.02	0.24	4.04
Jinshanpu (Indeterminate)	2.24	0.92	-0.09	-0.07	-1.94	1.09	0.87	2.80
Long79-6804 (Semicultivated)	11.47	1.32	0.24	-0.64	12.98	14.70	0.49	1.23
Lumeshido (Semicultivated)	4.44	1.07	0.13	-0.12	10.43	22.56	-3.11	-4.70

Semicultivated soybean Long79-6804 and Lumeshido have the highest positive gca on plant height, branches number, length of internode and have the lowest negative gca on seed-stem ratio and harvest index. On the other hand, the semideterminate soybean Hefeng 25 and Dongnong72-806 have the opposite tendency to those of semicultivated soybean. While the gca of those characters of indeterminate soybean Manchangjin and Jinshanpu are in the middle of these semicultivated and semideterminate soybeans.

Manchangjin and Jinshanpu have the highest positive gca in oil content and lowest negative gca in protein content, respectively. On the contrary, semicultivated soybean Long 79-6804 and Lumeshido have the highest positive gca on protein and lowest negative gca on oil content. In soybean breeding procedure, the effect of the three types of soybean on the main agronomic characters is obvious. Semicultivated soybean have promoting effect on plant height, node and branch number and protein content, but have reducing effect on seed-stem ratio, harvest index and oil content. In contrast, semideterminate soybean have promoting effect on seed-stem ratio, harvest index and have reducing effect on plant height and length of inter-nodes. Mangchangjin and Jinshanpu have promoting effect on oil content. Based on the results presented, crossing semicultivated soybean with semideterminate cultivated soybean could reduce plant height and increase seeed-stem ratio of progenies, effectively.

The comparison of the mean value and combining ability on main agronomic characters for different types of crosses: In the crosses within the same type of soybean, cross of semideterminate x semideterminate have the lowest mean value, specific combining ability and total combining ability in seeds and pods number and seed yield per plant, respectively. In the cross between different type of soybean, cross of semideterminate x semicultivated have the highest and the cross of semideterminate x indeterminate x have the lowest specific combining ability and mean value on the seed and pod number and seed yield per plant (Table 5). Those results indicate that there are significant differences of specific combining ability on the main yield characters among different types of crosses. In addition, the order of specific combining ability on main yield characters for different types of crosses is the same as that of mean value. It is suggested that we should pay more attention to the specific combining ability in the main yield characters in soybean breeding procedure.

Table 5. The breeding value, specific combining ability, total combining ability and mean value of the main yield and quality characters for different crosses of F_1 generation

The type of crosses	Parameter	Pods per plant	Seeds per plant	Yield per plant	Protein content	Oil content
Semideterminate x Semideterminate	Breeding Value	-22.43	-24.82	-2.14	1.23	0.48
	Specific Combining Ability	-20.22	-44.90	-3.29	0.68	0.58
	Total Combining Ability	-42.65	-69.72	-5.43	1.91	1.07
	Mean Value	53.76	149.67	25.75	41.10	20.85
Indeterminate x Indeterminate	Breeding value	-22.20	-22.13	4.25	-1.91	0.65
	Specific Combining Ability	12.14	23.03	2.95	-1.12	0.63
	Total Combining Ability	-10.06	0.96	7.20	-3.03	1.28
	Mean Value	84.00	208.53	33.95	36.17	21.11
Semicultivated x Semicultivated	Breeding value	34.67	46.95	3.60	0.67	-1.15
	Specific Combining Ability	2.89	-23.86	2.82	0.82	-0.49
	Total Combining Ability	37.56	23.09	0.78	1.49	-1.65
	Mean Value	137.67	219.60	31.95	40.68	18.14
Semideterminate x Indeterminate	Breeding value	22.32	-20.21	1.06	-0.34	-0.23
	Specific Combining Ability	2.74	-0.51	-0.62	0.32	0.08
	Total Combining Ability	19.57	-20.72	0.43	-0.02	-0.15
	Mean Value	75.25	137.47	29.49	39.17	19.96
Semideterminate x Semicultivated	Breeding value	11.11	26.52	0.73	0.95	-0.34
	Specific Combining Ability	7.37	22.96	2.26	-0.65	0.03
	Total Combining Ability	18.48	34.03	2.99	0.13	-0.20
	Mean Value	114.41	238.96	34.76	39.49	19.59
Semideterminate x Semicultivated	Breeding value	11.62	23.84	3.93	-0.62	-0.24
	Specific Combining Ability	-8.82	-11.03	-0.85	0.07	0.03
	Total Combining Ability	2.80	12.80	3.07	-0.55	-0.21
	Mean Value	98.33	209.79	32.34	38.82	19.68

Table 6. The breeding value, specific combining ability, total combining ability, and mean value of the main agronomic morphological characters for different crosses of F_1 generation

The type of crosses	Parameter	Plant Height	Branch Number	Height Between Nodes	Seed Stem ratio	Harvest index
Semideterminate x Semideterminate	Breeding Value	-18.40	-2.61	-0.58	0.69	0.05
	Specific Combining Ability	-7.22	-0.47	0.01	0.47	0.03
	Total Combining Ability	-25.43	-3.08	-0.57	1.16	0.08
	Mean Value	58.73	-0.67	3.95	2.74	0.55
Indeterminate x Indeterminate	Breeding Value	2.48	1.21	-0.12	0.11	0.01
	Specific Combining Ability	2.66	-0.35	0.02	0.16	0.03
	Total Combining Ability	5.14	0.86	0.14	0.05	0.04
	Mean Value	89.47	4.60	4.65	1.63	0.48
Semicultivated x Semicultivated	Breeding Value	15.87	1.39	0.47	-0.67	-0.06
	Specific Combining Ability	-7.69	-0.40	0.02	0.15	-0.03
	Total Combining Ability	8.18	0.99	0.49	-0.42	-0.09
	Mean Value	92.47	4.73	4.98	1.17	0.38
Semideterminate x Indeterminate	Breeding Value	-7.96	-0.70	-0.23	0.29	0.03
	Specific Combining Ability	-0.81	0.11	-0.01	-0.08	-0.01
	Total Combining Ability	-6.57	-0.60	-0.24	-0.21	0.02
	Mean Value	76.23	2.90	4.28	1.75	0.49
Semideterminant x Semicultivated	Breeding Value	-1.25	-0.60	-0.13	0.08	-0.01
	Specific Combining Ability	4.41	0.13	0.08	-0.96	-0.02
	Total Combining Ability	3.16	-0.47	-0.05	-0.90	-0.03
	Mean Value	87.36	3.77	4.46	1.53	0.47
Indeterminate x Semicultivated	Breeding Value	10.00	5.80	0.30	-0.35	-0.03
	Specific Combining Ability	1.68	0.70	0.05	-0.44	-0.01
	Total Combining Ability	11.66	1.44	0.35	-0.79	-0.04
	Mean Value	93.00	5.12	4.8	1.28	0.46

In all crosses, cross of Manchangjin x Jinshanpu have the highest and cross of Long79-6804 x Lumeshido (semicultivated x Semicultivated) have the lowest mean value, breeding value and total combining ability in oil content. The protein content has the opposite tendency to the oil content (Table 5). Manchangjin is a well known cultivar for its high oil content, Jinshanpu has higher oil content, too. Our study has also verified the productive situation.

From Tåble 6 it can be seen that in the crosses within the same type of soybean, crosses of semideterminate x semideterminate have lowest mean value, breeding value and total combining ability in the plant height, branch number and length between nodes and have highest seed-stem ratio and harvest index. Cross of semicultivated x semicultivated has the opposite tendency. In crosses among different types of soybean, the mean soybean, breeding value and total combining ability on seed-stem ratio and harvest index has the tendency: semideterminate x indeterminate > semideterminate x semicultivated > indeterminate x semicultivated, but plant height, branch number and length between nodes have the opposite close relationship with the mean value and combining ability on the main morphological characters. As the soybean parents evolved from lower to higher level, the mean value, breeding value and total combining ability on plant height, branch number, length between nodes of their offsprings decreased from higher to lower, but the harvest index and seed-stem ratio have the opposite tendency. In soybean breeding procedure, we should take the breeding value in morphological characters as an important selecting objective.

Conclusion: Previously, we only knew that the semicultivated soybean had higher plant height and more luxuriant growth. Moreover, by crossing semideterminate soybean with the lower evolutionary level soybean germplasm, could easily obtain erect plant in the progenies. But this has not been fully understood. On the results presented, we suggest that the infusing of semicultivated germplasm on the cultivated cultivars, could effectively increase the progeny's pod and seed number. Therefore, the semicultivated soybean germplasm is a very valuable germplasm to increase yield potential in soybean yield improvement. Semideterminant soybean could effectively reduce the plant height, length between nodes and increase the seed-stem ratio. Semideterminate x semicultivated is a promising combination of introducing genetic source of semicultivated soybean to cultivated soybeans.

References:

Cheng, Henghe. 1982. The diallel analysis in soybean main quantitative characters. Science in soybean. 1:41-52.

Gai, Junyi. 1983. The combining ability and hybrid vigor of crosses between American and China cultivars in F_1 and F_3 generation. Science in soybean 2:183-189.

Kaw, R.N. 1981. Combining ability in soybean. Indian of Genet. Breeding. 1981:40, 1:305-309.

Kunta, T. 1985. Heterosis performance and combining ability in soybean. Soybean Genet. Newsl. 12:97-99.

Ohm, H.W. 1973. Estimation of combining ability, hybrid vigor and gene action for protein in Avena spp. Crop Sci. 13:55-58.

Pascal, E.H. et al., 1975. Heterosis and combining ability in soybean germplasm. Crop Sci. 15:345-348.

Srirastrava, R.L. 1978. Combining ability for yield and related attributes in soybean. Indian Journal of Agric. Sci. 48:143-155.

Yang Qi
Wang Jinling

OIL CROPS RESEARCH INSTITUTE
Chinese Academy of Agricultural Sciences
Wuhan 430062, P.R. of China

Identification of soybean genotypes in Southern China for symbiotic characters

In Southern China,soybean production areas are vast in territory with diverse, favorable conditions of light, temperature, water, soil, and complex cropping systems. The soybean germplasm adapted to the conditions has genetic diversity (Xu Qiaozhen, 1990), including symbiosis (Zhang Xuejiang and Jiang Mulan, 1993). The study, which was a part of the subject supported by National Science Foundation of China and IBPGR, was to identify 200 accessions of spring, summer and autumn soybean genotypes from provinces in Southern China for agronomic, phenological and symbiotic characters, to statistically analyze correlations between symbiotic characters and agronomic or phenological characters, and to evaluate soybeans with improved N_2 fixation for production use and research.

Materials and Methods:

Table 1. Cultivars tested

Type	Source							Total
	Hubei	Hunan	Jiangsu	Guangdong	Zhejiang	Fujian	Yunnan	
Spring	32	9	12	2	7	0	0	62
Summer	90	1	8	13	0	0	1	113
Autumn	0	3	0	0	12	10	0	25
Total	122	13	20	15	19	10	1	200

200 accessions of spring, summer and autumn soybean germplasms collected from seven provinces in Southern China are listed in Table 1. These cultivars were used in field experiments, and 100 of the cultivars in greenhouse were inoculated with Bradyrhizobium japonicum strain 113-2.

The field experiment was arranged at the Experiment Farm of the Oil Crops Research Institute, with plots of three rows, three m long. Plants x rows to be 10 x 40 (cm), 10 x 50 (cm) and 10 x 40 (cm) apart for spring, summer and autumn soybeans, respectively.

The greenhouse experiment was conducted by water pot culture with N-free nutrient solution (Zhou Pingzhen et al., 1979). The cultivars were

inoculated with strain 113-2 and uninoculated control. The pots were laid out in a randomized complete block design with four replications.

The method for evaluating N_2 fixation (analysis of total N in plant and seed by kjeldhl's, and ureide by xylem-solute technique) described as Peoples et al. was used. The amount of N_2-fixed is calculated as:

1.) N_2-fixed (mg/plant) = N inoculated-N uninoculated

2.) % of N_2 fixation = N_2-fixed/ N inoculated x 100 and total ureide contents of sample were determined from curve prepared from optical densities of allantoin standards, and a correction factor used (ie; 0.05 ml samples used factor is 1.0/0.05 = x 20) to convert sample μ mole determinations to μ mole/ml.

Experimental data were as follows:

Agronomic characters:

1.) Productivity (G/plant) 2.) 100 seed weight (g) 3.) Plant height (cm/plant) 4.)Node number/plant 5.) Branch number/plant 6.)Blighted pods/plant 7.)Total pods/plant.

Phenological characters:

8)The days of emergence seedling to flowering 9)The days of flowering to maturity 10)The days of duriation (emergence seedling to physiological maturity).

Symbiotic characters - 11.)Nodule number/plant 12.)Nodule weight (g/plant) 13)Dry weight (g/plant) 14)Total plant N (mg/plant) 15)Seed protein (%) 16)Total seed N (mg/plant).

Results and Analysis: The field experiment indicated (Table 2) that:

Agronomic characters - All the character variations of summer soybeans were more than that of spring or autumn. It was summer > autumn > spring in productivity per plant and branch number number, summer > spring . autumn in plant height, node number, and pod number, except 100 seed weight.

Phenological characters - Also, the character variations of summer soybeans were the highest, with summer > autumn > spring in the days of duriation and flowering to maturity, and summer > spring > autumn in the days of emergence seedling to flowering.

Symbiotic characters - yet, the variations of the characters, nodule number, nodule weight, plant weight, total plant N, seed protein and total seed N of summer soybeans were greater than that of either spring or autumn soybean.

The difference between maximum and minimum value was summer > autumn > spring.

Besides, correlation analysis for symbiotic characters with agronomic/phenological characters of different soybean types indicated that the correlation coefficient was summer>spring>autumn on the whole. Variance analysis indicated that the significant difference ($p<0.05$ oe $p<0.01$) was present in different cultivars from the same soybean type tested for agronomic, phenological and symbiotic characters. Some soybean genotypes available for use in breeding as parents for improving N_2 fixation and utilization in the crop production, have been evaluated from the 200 accessions, like a genotype, 80-1041, has been used in production.

Table 2. Identification of spring, summer and autumn soybeans for agronomic (1-7), phenological (8-10) and symbiotic (11-16) characters.

Type	1		2		3		4	
	a	b	a	b	a	b	a	b
Spring	4.84	8.10	12.73	11.70	41.10	64.60	13.20	8.60
Summer	10.07	18.50	14.52	26.70	74.40	188.80	19.67	22.20
Autumn	8.65	11.50	21.35	34.60	41.82	32.80	13.00	5.40

	5		6		7		8	
	a	b	a	b	a	b	a	b
Spring	3.2	4.6	1.5	8.4	30.9	61.6	44.2	24
Summer	4.7	9.4	9.7	140.5	79.5	205.4	50.1	43
Autumn	3.8	8.8	3.0	8.0	28.8	48.2	34.8	15

	9		10		11		12	
	a	b	a	b	a	b	a	b
Spring	50.5	25	94.4	39	21.7	58.0	0.192	0.425
Summer	74.0	66	128.6	87	47.3	200.6	0.243	1.550
Autumn	60.9	26	98.9	16	14.0	35.0	0.068	0.274

	13		14		15		16	
Spring	a	b	a	b	a	b	a	b
Summer	3.81	6.68	103.31	202.20	43.25	10.25	315.15	548.95
Autumn	13.35	34.88	357.94	900.76	45.80	12.72	735.07	1268.85

a-Average value
b-Difference = maximum - minimum

The greenhouse experiment results of 1992 and 1993 similarly showed (table 3) that significant differences ($p<0.05$) existed among the types; All of nodule number, top weight, N_2-fixed proportion of N_2 fixation and ureide was summer = autumn > spring. These were similar to the results from field experiment.

Table 3. Nodulation and N_2 fixation by the three types

Type	No. of cultivars tested	Nodules /pl	Top Weight (g/pl.)	N_2-fixed (mg/pl.)	N_2-fixation (%)	Ureide (μmole/ml)
			1992			
Spring	16	27.3^b	104^b	14.00_b	33.0^b	1.01^b
Summer	29	36.1^{ab}	1.70^a	49.41^a	64.0^a	2.14^a
Autumn	5	47.7^a	2.34^a	59.99^a	71.0^a	1.40^b
			1993			
Spring	16	24.0^b	0.97^b	17.77^b	35.3^b	0.87^b
Summer	28	29.0^{ab}	1.64^a	41.74^a	45.1^{ab}	1.46^a
Autumn	6	34.0^a	2.15^a	55.47^a	57.0^a	1.32^a

a, b - Significant difference at $p=0.05$

Statistical data indicated that the parameters of symbific activity were significantly correlated with each other and that significant differences ($p<0.05$

or $p<0.01$) of the symbiotic occurred in both different types and different cultivars. Some cultivars with improved nodulation and nitrogen fixation have been obtained from 100 accession germplasms. The results were close to those from field experiment. Two of these cultivars, TaiXinHei and AiJiaoZao, have been used in genetic analysis and breeding program.

References:

Peoples, M.B. et al. 1979. Methods for evaluating nitrogen fixation by nodulated legumes in the field. Tampworth. NSE. Australia.
Xu Qiaozhen. 1990. Oil Crops of China. 4:38-42 (in Chinese).
Zhang Xuejiang and Jiang Mulan. 1993. Current developments in soybean - Rhizobium symbiotic nitrogen fixation (ed. Dou Xintian). p. 85-102. Heilongjiang Science & Technology publishing house. Harbin.
Zhou Pingzhen. 1979. OII Crops of China. 2:60-62. (in Chinese).

Xu Qiaozhen
Zhang Xuejiang
Jiang Mulan
Li Zhiyu

INSTITUTE OF OIL CROPS, CAAS
Wuhan 430062
CHINA

Genotype by Environment Interactions of Tofu Processing Traits in Soybeans

Abstract: The tofu processing traits of soybean lines in two regional testings were analyzed. The ratios of finished tofu were different among those lines and locations. The line X location variance of those traits were significant at 1% probability level and genotype by environment interactions were observed, but the stability of various lines was different. It is possible to improve the stability of cultivars in tofu processing quality.

Key Words: [Glycine max (L.) Merr.], Tofu processing, Genotype by environment interaction.

The importance of the genotype by environment interaction of seed quality and the stability of variety were discovered with researching on the improvement of soybean seed quality. Schutz and Bernard (1) estimated the interaction variance for the Uniform Soybean Tests, Maturity groups 0 to IV, VI and VII. They considered that for protein and oil, the interaction components were much less than one for yield. Kwon and Torrie (2) evaluated F3, F4 and F5 generations of lines from two crosses. In both populations, the variance components for lines was greater than the interaction components for all traits except yield. The line X year variance components were larger than either the line X location or line X location X year components for percent oil. The three way interaction was more similar in size to the line X year interaction than the line X location for all traits except flowering, fruiting period, and percent protein. The larger line X year effect was thought to be a result of seasonal differences in rainfall and temperature. Erikson et al. (3) determined percent protein for 115 F3 lines from crosses between Glycine max and G. soja in 2 yrs and two locations. As in the previous studies the line X location X year component was found to be significant and larger than either of the two-way interaction, but less than the variance components due to lines. Zhang et al. (4) studied the fatty acid compositions of 6 varieties in 2 yrs and 14 locations. They found that the fatty acid compositions varied with the change of locations and years, but different varieties and compositions had different stability to environment.

There were many reports on genotype by environment interaction of yield and agronomic and quality characters, but not reports on that of tofu processing

traits in soybeans. The object of this thesis was to study the genotype by environment interactions of ratio of finished tofu in the regional testing of soybeans and study the use of this interaction in soybean improvement and utilization.

Material and Methods:

A. Experimental material and field test

1. Experiment I: Seven varieties or lines in Hubei Province Spring Soybean Regional Testing (Mid-season Group) were used in seven locations in 1992.
2. Experiment II: Seven varieties and lines in South of China Spring Soybean Regional Testing (Mid-season Group) were used in nine locations in 1992.

The field tests in various locations were to be arranged on random block with three replications. The area of every plot was 6.67m^2.

B. Analysis of the tofu processing:

The seeds of every plot in all locations were collected and the ratio of finished tofu was determined on the tofu processing method of small sample (the seed weight was 30 gram) [5] in the Institute of Oil Crops, CAAS. The yield of dry tofu per unit area was calculated on the seed yield of plot and ratio of dry tofu.

C. Statistical treatment of data:

All statistical analyses were made on a mixed model to line and location and the significance of variances for lines, locations and line X location were tested [6]. The correlation coefficient between environment index which was mean seed yield of various location and ratio of dry tofu was calculated as well as the response of soybean varieties to environment.

Experiment Results: 1. The genotype by environment interaction of ratio of finished tofu:

The results (in Table 1) indicated that line variance and location variance were significant in four characters in two experiments, except the variance for lines of the ratio of wet tofu in Experiment 1. The line X location variance components were also significant at 1% probability level. The location variance component was larger than line or line X location component, except ratio of wet tofu in Experiment II. For example, the location variance of ratio of dry tofu in Experiment I was 11.96, the line and line X location variance was 1.57 and 0.78, respectively. The results showed that the ratio of finished tofu varied with

the location, and the genotype by environment interactions were existent, illustrating the need for testing the tofu processing characters in multiple environment.

The Experiment II was located in five province and Experiment I located only in Hubei Province, but the location variances in Experiment I were also significant. They indicated that the ratio of finished tofu was very susceptible to environment.

Table I. Genotype X environment interaction variance of some characters in soybeans.

source	ratio of wet tofu	ratio of dry tofu	seed yield	dry tofu yield
Experiment I				
lines	11.93	1.57**	0.61**	0.19**
locations	209.03**	11.66**	1.37**	0.39**
line X locat	21.95**	0.78**	0.20**	0.05*
error	7.37	0.26	0.019	0.005
Experiment II				
lines	98.41**	1.98**	0.18**	0.02**
locations	36.62**	3.49**	2.50**	0.28**
line X locat	22.62**	1.07**	0.10**	0.01**
error	11.54	0.42	0.01	0.0015

**-- significant at 1% probability level.

2. Environmental stability of varieties:

The results showed in table 2 indicated that the environmental coefficients of variation (ECV%) of ratio of finished tofu were less than yield, and were different among the varieties. For example, ECV% of Aijiaozao in two experiment was least among all varieties, and ECV% of En86-49 was largest. It showed that it was possible to improve the environmental stability of variety.

Table 2. Environmental coefficients of variation (ECV%) of various lines in soybeans.

lines	ratio of wet tofu	ratio of dry tofu	seed yield	dry tofu yield
Experiment I				
Enong 89-3	5.05	5.73	16.03	20.05
Mian 89-1	7.36	5.72	28.48	26.45
Aijiaozao	4.51	5.35	25.92	28.21
Mian 90-7	7.69	5.66	21.90	22.20
You 90-10	6.17	5.22	23.01	21.26
En86-49	8.35	4.59	27.88	31.70
You 90-8	4.36	7.40	15.15	19.52
Experiment II				
Aijiaozao	2.39	4.21	28.99	29.99
Xiangchen13	3.21	3.60	34..54	37.14
En 86-49	6.25	6.00	39.91	42.97
Lin 86-2	5.09	4.97	23.68	26.90
Zhe 8839	4.74	5.60	28.64	29.72
Sou 8802	4.37	3.41	39.31	39.50
Xiangchen10	5.96	3.75	23.42	26.40

3. Response of various varieties to change of environment:

The correlation coefficients between environmental index and ratio of finished tofu were shown in Table 3. The results indicated that there was better response in seed yield of soybean variety to better environment, because the correlation coefficients between environmental index and seed yield were positive and significant at 5% or 1% probability level. The response of various varieties in ratio of finished tofu to environment was different. Most of these varieties had a positive response, but there were only two varieties in experiment II whose correlation coefficient between environmental index and ratio of dry tofu was significant at 5% probability level. In addition, this response of same variety was different in two experiments.

Table 3. Correlations of quality of processing and environmental index in soybean.

lines	ratio of wet tofu	ratio of dry tofu	seed yield	dry tofu yield
Experiment I				
Enong 89-3	0.23	0.53	0.96**	0.90**
Mian 89-1	0.16	-0.13	0.698	0.76*
Aijiaozao	-0.05	0.10	0.79*	0.76*
Mian 90-7	0.65	0.31	0.76*	0.82*
You 90-10	0.49	0.09	0.72*	0.79*
En 86-49	0.20	0.31	0.68	0.640
You 90-8	0.43	0.37	0.94**	0.86**
Experiment II				
Aijiaozao	0.41	0.75*	0.95**	0.98**
Xiangchen13	0.27	0.71*	0.97**	0.98**
En 86-49	0.52	0.03	0.77**	0.80**
Lin 86-2	0.41	0.42	0.94**	0.94**
Zhe 8839	-0.19	0.24	0.97**	0.93**
Sou 8802	-0.24	0.35	0.88**	0.88**
Xiangchen10	0.36	0.41	0.98**	0.97**

* or ** -- significant at 5% or 1% probability level.

Discussion: In general, the variance for variety or line was larger than variance for location or variance for line X location in the previous studies. Because of the experimental material and location, the results in this research were opposite, but the variance of tofu processing quality for variety were very significant. This indicated that there were significant differences. In addition, the line X location variance components were significant, the genotype by environment interaction of tofu processing quality were existent.

Although there were genotype by environment interactions, the environmental stability in tofu processing quality was different in varieties, and it would be possible to improve this quality and its stability. In addition, the difference in varieties or location could be used to produce commercial soybean which had better tofu processing quality.

References:
Schutz, W.M. and R.L. Bernard. 1967. Genotype X environment interaction in the regional testing of soybean strains. Crop Sci 7:125-130.
Kwon, S.H. and J.H. Torrie. 1964. Heritability of the interrelationships among traits of two soybean populations. Crop Sci. 4:196-198.
Erikson, L.R.,et al. 1982. Genotype X environment interaction for protein in Glycine max X Glycine soja crosses. Crop Sci 22:1099-1101.
Zhang Guodong et al. 1990. Varietal and Environmental Variations of fatty acid compositions in soybean seed oil, in "Advance of Basic and Technological Aspects in Breeding for soybean", pp 132-128. Jiangsu Science and Technology Publishing House, China.
Zhou Xinan, Gai Junyi and Ma Yuhua, 1992. Variation of tofu processing traits and their relationships with component contents of seed storage protein in soybean, Soybean Science (Chinese), 11 (4):283-289.
Ma Yuhua. 1982. Basic of Biometrical Genetics in Plant Breeding, Jiangsu Science and Technology Publishing House, China.
Bilbro, J.D. and L.L. Ray. 1976. Environmental stability and adaptation of several cotton cultivars. Crop Sci. 59(3):685-689.

Zhou Xinan
Zhu Jiancha

INSTITUTE OF BOTANY
Academia Sinica
Nankang, Taipei 115
Taiwan, Republic of China

Polymorphic distinction of soybean by molecular markers.

Introduction: Molecular markers are believed to be an efficient tool to assist plant improvement (Tanksley, 1983; Paterson et al., 1991; Dudley, 1993). Presently, at least three types of molecular markers i.e. isozymes, RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) are commonly used. The major drawback of isozyme assay is its being limited in numbers of loci. Our previous study of soybean RFLP on 20 selected Taiwan's soybean breeding resources indicated that soybean probes, which are believed to be primarily single copy sequence derived from genomic library of *Pst*1 digestion (Keim and Shoemaker, 1988), are a very good resource for detecting polymorphism among screened accessions. Nevertheless, the RFLP approach is time consuming and labrious in comparison with others. Recently, RAPD markers, derived from arbitrarily primed polymerase chain reaction, are reported to be another potential DNA markers(Williams et al., 1990; Newbury and Ford-Lloyd, 1993).

The objective of this study is to reveals the relative efficiency and frequencies of polymorphic loci with a close number of assayed enzymes, probes and primers by the above three methods based on assaying 20 selected soybean accessions.

Materials and Methods: Twenty soybean accessions, including three local varieties, four improved cultivars, eight breeding lines or plant introduction, two pairs of isogenic lines with difference in seed coat color and one Glycine soja accession were included for detecting polymorphisms. In isozyme assay, 15 enzymes and one protein, i.e. AP (acid phos-phatase), ADH (alcohol dehydrogenase), -AM (- amylase), DIA (diaphorase), ENP (endopeptidase), EST (esterase), EU (urease), GOT (glutamate oxaloacetic trans-aminase), IDH (isocitric dehydrogenase), LAP (leucine aminopeptidase), MDH (malate dehydrogenase), 6-PGD (6-phosphogluconate dehydrogenase), PX(peroxidase), SDH (shikimic dehydrogenase), XDH (xanthine dehydrogenase) and trypsin inhibitor (TI) were analyzed following Chen et al., 1989; For RFLP study, 17 probes derived from Pst1 genomic library were used

and a total of 53 probe-enzyme combinations were tested (data from Chen et al., 1993); and in RAPD screening, 20 decamers (Kit OPF) from Operon Technologies (Alameda, California) were primed for the detecting of polymorphic DNAs. Total DNA was extracted from soybean leaf according to Chen et al (1993). The PCR reaction components were 50 mM KCl, 10 mM Tris-HCl pH 8.3, 200 μM each of dNTP (dATP, dCTP, dGTP and dTTP), 1.0 μM primer, 2.5 Units AmpiTag DNA polymerase, templete DNA 100-200 ng and 3.0 mM $MgCl_2$ for each 100μl reaction volume. The temperature cycling program is 94 C (1min.) - 36 C (1min.) - 72 C (2min.) for 45 cycles. The amplified products were then run in a 2% agarose gel and stained with ethidium bromide (0.05%).

Cluster analysis based on average linkage (UPGMA) model was conducted with coefficient of genetic similarity estimated by these three types of marker loci individually.

Results and Discussion: From the 20 selected soybean accessions, it is indicated that molecular markers derived from different sources (i.e. isozymes, RFLPs and RAPDs) all revealed polymorphisms among the accessions. However, difficulty in distinguishing isogenic lines remains for all markers tested. As shown in Table 1, 10 out of 16 (62.5%) enzymes revealing polymorphisms among soybean accessions; 11 out of 17 (64.7%) soybean probes were capable of revealing RFLPs in at least one restriction enzyme digestion, but from a total of 53 probe-enzyme combinations only 28.3% revealed polymorphisms; 11 out of 20 (55%) primers indicated RAPD polymorphisms among the 20 soybean accessions. Numbers of polymorphic loci obtained from this study were 10, 25 and 17 for isozyme, RFLP and RAPD, respectively.

Results from cluster analysis indicates RAPDs can reveal more cluster groups than RFLPs and isozymes, nevertheless, the three markers behave somewhat differently in grouping (Table 2). This might suggested that genetic distribution of these isozyme, RFLP and RAPD markers used in this study might be quite different in soybean and they should be used complementally.

A brief comparison was made among three methods (Table 3). Although isozyme approach is the most rapid and easy way for assay, limitation in numbers of enzymes which are capable for analysis and polymorphic loci available are the main drawbacks. RFLP analysis has the advantages of generating abounding polymorphic loci but is the most time consuming and

labourious method among the three. RAPD has the relative advantage in both number of polymorphic loci and the automation in PCR cycling, however, caution is needed for sample preparation and reaction parameters in order to make the result reproducible.

Apparently, each type of molecular makers has its own drawbacks. Strategy in applying molecular makers should be taken to make it more efficiently.

References:

Chen, L.F.O., G.C. Chen, S.F. Lin and S.C.G. Chen. 1993. Polymorphic differentiation and genetic variation of soybean by RFLP analysis. Bot. Bull. Acad. Sinica 34:249-259.

Chen, L.F.O., W.C. Hu and S.C.G. Chen. 1989. Analysis of zymogram variations on cultivated soybean(*Glycine max* L. Merr.) of Taiwan. Bot. Bull. Acad. Sinica. 30:179-190.

Dudley, J.W. 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. Crop Sci. 33:660-668.

Keim, P. and R.C. Shoemaker. 1988. Construction of a recombinant DNA library that is primarily single copy sequences. Soy. Genet. Newsl. 15:147-152.

Newbury, H.J. and B.V. Ford-Lloyd. 1993. The use of RAPD for assessing variation in plants. Plant Growth Regulation 12:43-51.

Paterson, A.H., S.D. Tanksley and M.E. Sorrells. 1991. DNA markers in plant improvement. Adv. Agronomy 46:39-90.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Mol. Biol. Rep. 1:3-8.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18(22):6531-6535.

Long-Fang O. Chen
Wen-Chu Yun,
Hsiao-Yin Kuo
Mei-Hwei Chen

Table 1: Polymorphic frequencies among types of molecular markers in soybean

Types of Molecular marker	No. of Enzymes, Probes or Primers	Polymorphic Enzymes , Probes, or Primers	Polymorphic frequencies (%)	No. of Polymorphic.... Alleles	Polymorphic Loci. estimated
Isozyme	16	10	62.5	26	10
RFLP	17	11	64.7	43	25
	(53)[a]	(15)	(28.3)	-	-
RAPD	20	11	55.0	17	17

a) Value in the parentheses is estimated based on No. of Probe-enzyme combinations screened.

Table 2. Differences in grouping of cluster analysis based on similarity of isozyme, RFLP or RAPD patterns of 20 selected soybean acessions

Accessions	Grouping		
	Isozyme	RFLP	RAPD
AGS-129	III	I	VII
HCWT	II	III	I
FWCP	V	I	VI
TN15	III	III	III
HL-1	III	III	VIII
KS-10	III	I	VII
G2120	II	I	VI
PI245331	V	V	V
SKT	III	II	I
BPCL	III	II	I
TEKKYOKERO	I	I	II
TA92785	I	II	IV
TA92616	IV	III	IV
TA93005	II	IV	VIII
TA93066	I	II	IV
TA93235	IV	II	I
L87B	I	I	I
L87Y	I	I	I
L94B	I	I	I
L94Y	I	I	I

Table 3. Comparisons on time consuming among analysis processes of three types of molecular markers routinely used in our laboratory

Items	Isozyme	RFLP	RAPD
Plant growth	0	2-3 weeks	2-3 weeks
Enzyme or DNA extractions	sucked overnight & extract <30min	1.5 days	1.5 days
Gel electrophoresis	5-10 hrs	overnight	7 hrs (f r 15cm gel)
Activity staining	0.5 hrs- overnight	-	10min
P.C.R reaction	-	-	4-5 hrs
Plasmid DNA isolation	-	1.5 days	-
DNA digestion	-	4-5 hrs	-
Southern blotting	-	16 hrs	-
Hybridization	-	1.5 days	-
Autoradiography	-	2-4 days	-
Minimum time required from sample extraction to obtain results	< 2 days	9-11 days	< 3 days
Replication	3X (& 2-3 enzymes at a time)	1X	2X
Detectable sources	Limited	Many	Almost unlimited

INSTITUTE OF BOTANY
Academia Sinica,
Nankang ,Taipei 115
Taiwan, Rep. of China

Differential amplification between root and leaf DNAs in soybean by RAPD markers

Introduction: Random amplified polymorphic DNA (RAPD) derived from AP-PCR (arbitrarily primed chain reaction)(Welsh and McClelland, 1990; Williams et al., 1990) is one of the efficient molecular markers. Presently, the RAPD method is mostly used for genetic fingerprinting , species and cultivar identification or genetic mapping studies. In plant , rapid genomic changes involving chromosome rearrangements, chromosome imprinting, gene amplification, loss and transposable elements have been addressed during the life span (McClintock, 1984; Walbot and Cullis, 1985). Whether the plant genome is capable of alternation under a changing environment or during the developmental stages is questioned. In recent years, molecular approaches to the genes which regulate plant and organ development have been in progress (Aeschbacher and Benfey, 1992; Lyndon and Francis, 1992). Young (1993) pointed out DNA markers can be applied for studying the genetics of plant growth and development. Changes in genome organization that occur during development or in response to environmental signals can be monitored by DNA markers. In this communication, we report the difference in RAPD patterns between root and leaf DNAs of soybean grown under hydroponic culture.

Materials and Methods: A total of 11 soybean lines, representing 2 local land varieties of Taiwan, two cultivated varieties, 2 pairs of isogenic line derived from F5 selfed progeny, and three plant introductions were obtained from Taiwan Agricultural Research Institute. Seeds of soybean were germinated and grown under hydroponic culture (Barrentine et al., 1976) at 28° C day (16hr)/ 24° C night (8hr) temperature in a growth chamber.

The procedures for sampling and DNA isolation are essentially similar to that of Chen et al., (1993) except root and leaf tissues are collected separately. The extracted DNAs are further diluted to 100 ng/µl with TE (10:0.1) and served as template DNA for polymerase chain reaction (PCR).

The primers used for this study included sequencing primers (M13, M13R, KS17, SK17 and T7), oligonucleotide synthesized decamers [including four from Williams et al., (1990) and 20 OPF decamers from Operon Technologies Inc. (Alameda, California)], and partial sequences obtained from some known genes such as soybean actin gene, soybean glycinin gene, pea rbcs gene, tobacco peroxidase gene and rice salt induced protein gene, etc. A Techne PHC-3 Dri-Block Cycler (Techne Ltd., UK) was used for temperature control and cycling. For those primers with 17-22 mers, three cycles at 94° C for 5 min, 40° C for 5 min and 72° C for 5 min were initiated with 40 cycles of 94° C for 1 min, 50° C for 1 min and 72° C for 2 min for denaturing, annealing and primer extension, respectively. For all the decamers, 45 cycles of 94° C for 1 min, 36° C for 1 min and 72° C for 2 min were programmed. The reaction components in the PCR reaction and agarose gel electrophoresis are the same as our previous report (Chen et al.,1994). In addition to polymorphic differences among accessions, pairwise comparisons between the amplified products of leaf DNA and root DNA were also conducted.

Results and Discussion: From a total of 40 primers used, it was indicated that about 47.5% of these primers are capable of detecting polymorphisms among the 11 selected soybean lines (Table 1). Fourteen primers had shown differences in number of amplified fragments between leaf and root DNA samples in at least some accessions (Table 1). Variation in band intensity is also noted in some fragments (Fig.1B). Since RAPD markers are generally inherited as dominant type alleles in present or absent fashion (Williams et al., 1990), we hereafter consider each polymorphic band as a polymorphic locus. Among the polymorphic loci found, only one locus is leaf specific; 53% amplified band patterns found only in root DNA and about 46% of the polymorphic band patterns occurred in both leaf and root DNA amplifications . Nevertheless, instability of some bands was also observed. In this study , about 86% of amplified bands showed the inconsistency in pairwise comparison of leaf and root amplification (Table 2). Bands which are presented in leaf amplification are not necessarily found in the root amplification and vice versa . At least 21% of polymorphic bands occurred only in one or two soybean lines (Table 2). Polymorphic bands consistent in both leaf and root amplification are only found in about 12 loci. Whether the inconsistency between root and leaf amplification is a technical problem or due to genetic modification during leaf and root developments is under

study. DNA amplification in root hairs has been reported in *Hordeum vulgare* (Murry, et al., 1987). Plant materials are grown in hydroponic culture for the ease of root harvesting, whether hydroponic culture is a stress to soybean roots is not known. In soybean , genetic variation of tissue culture materials derived from root tissue of soybean had been revealed by restriction fragment length polymorphism study (Roth et al.,1989). They reported that tissue culture prepared from root tissue of individual soybean plants develop RFLP allelic differences at various loci and the generated alleles are almost always the same as ones previously found and characterized in other varieties of cultivated soybean . The difference between root and leaf DNA amplification might be an indication of developmental modification in soybean root genome or it might be induced under hydroponic stress. We believe that RAPD analysis should be a very good tool for detecting genomic modification during developmental stages or under stress, without the need of knowledge of target gene sequences.

References:

Aeschbacher, R.A. and P.N. Benfey. 1992. Genes that regulate plant development. Plant Science. 83:115-126.

Barrentine, W.L., C.J. Edwards, Jr. and E.E. Hwartwig. 1976. Screening soybean for tolerance to metribuzin. Agronomy J. 68:351-353.

Chen, L.F.O., W.C. Yun, H.Y. Kuo and M. H. Chen. 1994. Polymorphic distinction of soybean by molecular markers. Soybean Genet. Newsl. 21

Chen, L.F.O., K.C. Chen, S.F. Lin and S.C.G. Chen. 1993. Polymorphic differentiation and genetic variation of soybean by RFLP analysis. Bot. Bull. Acad. Sinica. 34: 249-259.

Lyndon, R.F and D. Francis. 1992. Plant and organ development. Plant Molecular Biology. 19:51-68.

McClintock, B. 1984. The signification of responses of the genome to challenge. Science. 226:792-801.

Murry, L.E., M.L. Christianson, S.H. Alfinito, and S.J. Garger. 1987. Characterization of the nuclear and of Hordeum vulgare root hairs: Amplification disappears under salt stress. Amer. J. Bot. 74(12):1779-1786.

Roth, E.J., B.L. Frazier, N.R. Apuya and K.G. Lark. 1989. Genetic variation in an inbred plant: Variation in tissue cultures of soybean [Glycine max(L.) Merrill]. Genetics. 121:359-368.

Walbot, V. and C.A. Cullis. 1985. Rapid genomic change in higher plants. Ann. Rev. Plant Physiol. 36:367-396.

Welsh J. and M. McClelland . 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research. 18(24):7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18(22):6531-6535.
Young, N.D. 1993. Applications of DNA genetic markers to study of plant growth and development. Plant Growth Regulation 12:229-236.

L.F.O. Chen
H.E. Kuo
S.F. Yang
S.C.G. Chen

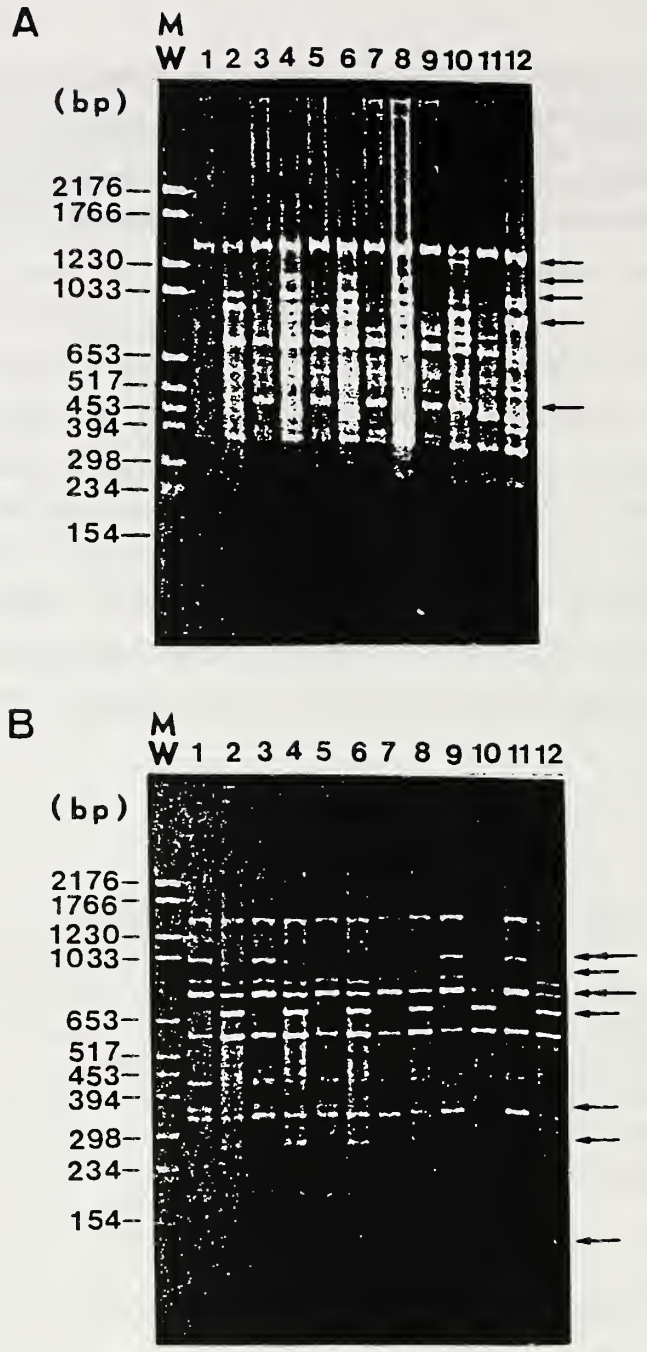


Figure Legend:

Figure 1. Pairwise comparisons of leaf and root DNA amplifications in RAPD patterns of six soybean lines. Lanes 1, 3, 5, 7, 9 and 11 are fragments of leaf amplifications in four isogenic lines with white (BB120W and BB105W) and purple (BB120P and BB105P) flower colors, one cultivated variety (AGS-58) and one Taiwan's land variety (HCWT), respectively. Lanes 2, 4, 6, 8, 10 and 12 are fragments of root amplifications of the above six soybean lines. (A) is amplified patterns derived from KS17 sequencing primer; (B) is derived from RSR2 primer. Arrows indicate specific amplification differences in leaf and root DNA. Fragments with variation in relative band intensity are pointed out with double arrows.

Table 1. Summary of primer sources, names and number of primer for detecting DNA polymorphisms in soybean AP-PCR study

Oligo-primer				
Source	Name	Total No.	No. of primer revealing leaf DNA polymorpisms	No. of primer indicating root and leaf difference
Sequencing primers	M13 ,M13R, KS17, SK17, T7	5	2(40%)	2(40%)
Known gene sequences	Act-22, Act1R-22, Soygy3, PRR1, Prr2, Rs1, kRSR1, RSR2, RSR3, TPXR1, TPXR2	11	3(27.3%)	3(27.3%)
Arbitrary primers	AP11, APW1, APW2, APG3, OPF-1 to OPF-20	40	19(47.5%)	14(35.0%)
	TOTAL	40	19(47.5%)	14(35.0%)

Table 2: Summary on numbers, patterns and percentage of polymorphic loci generated by RAPD analysis of root and leaf DNAs

	Primer Source			
Loci Classification	**Sequencing Primer**	**Arbitrary decamer**	**Known gene sequence**	**Total**
Polymorphic loci	38(38.0)[a]	40(40.0)	22(22.0)	100
Leaf Specific	0(0.0) (0.0)[b]	(0.0) (0.0)	1(100.0) (4.6)	1 (1.0)
Root Specific	27(50.9) (71.1)	18(34.0) (45.0)	8(15.1) (36.4)	53 (53.0)
Both root and leaf	11(23.9) (29.0)	22(47.8) (55.0)	13(28.3) (59.1)	46 (46.0)
Unstable[c] loci	36(41.9) (94.7)	32(37.2) (80.0)	18(20.9) (81.8)	86 (86.0)
Rare allele[d] loci	8(38.1) (21.1)	12(57.1) (30.0)	1(4.8) (4.6)	21 (21.0)
Monomorphic in leaf	32(47.8) (84.2)	18(26.9) (45.0)	17(25.4) (77.3)	67 (67.0)
Monomorphic in root	3(25.0) (7.9)	2(16.7) (5.0)	7(58.3) (31.8)	12 (12.0)

a: numbers within the () beside marked values indicate percentage against row total.
b: numbers within the () under marked values indicate percentage against total polymorphic loci found in each primer source.
c: loci with patterns of leaf and root samples are not consistent in some soybean lines.
d: allele is presented only in one or two soybean lines.

NORTHEAST AGRICULTURAL UNIVERSITY
China
and
NATIONAL INSTITUTE OF AGROBIOLOGY RESOURCES
Japan

Development of hybrids with higher susceptibility to *agrobacterium tumefaciens* A208 and inheritance of the susceptibility on soybeans

Introduction: Regard as the desired gene transformation vectors, many strains of A. tumefaciens are considered wide host-range pathogens on several dicotyledonous angiosperms and some gymnosperms, but the ability of the bacterium to form a compatible reaction varies widely among species. Most reports have proved that A. tumefaciens were avirulent or weakly virulent on soybean except nopaline-type strain A208 and C58(3), which was also confirmed in our previous work (unpublished). Recently, three genes have been identified in the T-DNA of nopaline-type strain to affect the formation of crown gall on plants. Those genes encode enzymes fo rauxin and cytokinin synthesis (1), and outside the T-DNA, at least six transcriptional loci clustered in the virulence region which controlled host range partially (3). Otherwise, host plant also possesses different sensitivity to agrobacterium due to endogenous phytohormone levels, cell wall fragments, quantity and type of phytoalexins (3).

Based upon the above review, it is possible to develop new superlines of soybean loaded with characteristics of higher susceptibility to supervirulent agrobacterium strain A208. Therefore, present experiment was conducted to develop higher susceptible hybrids of soybeans and evaluate agronomically important genetic parameters by constant parent regression and related techniques.

Materials and methods: 'Peking 501', which was identified as the most susceptible to agrobacterium A208 was selected as a constant parent and crossed with 'Ransom' (moderately susceptible), 'Wayne' (moderately susceptible), 'Akasaya 1' (weakly susceptible), 'American Jellow' (weakly susceptible), 'Bonminori' (non-susceptible) and Hill (non-susceptible), respectively. All F_0 seeds were planted in greenhouse. Three weeks after sowing, plants were inoculated by A. tumefaciens A208. Crown gall were scored after five weeks, including crown gall numbers (%) and size (mm). In this experiment, complete randomized design

was adopted with two replicates. Nopaline was analysed as described by Otten and Schiperoot (4).

Results: Following the procedure described in materials and methods, six combinations were tested for tumor inducting along F_1 plant stems. A significant difference was observed among these combinations on ratio of individuals forming crown gall (Table 1). The ratio values ranged from 49% to 100% (Fig. 1), indicating that the characteristics of agrobacterium A208/susceptibility in soybean was seemingly partially modified by multigenes. An attempt was undertaken to evaluate the mobilization possibility of genes highly susceptible to A208 from 'Peking 501', which has an unexpected trait of black seed coat, to other cultivars and to predict the breeding potential by constant parent regression technique.

Table 1 Variance Analysis of 6 combinations F1 hybrids

Factors	DF	V	F-value
Combinations	5	627.16	37.44++
Blocks	1	43.42	2.59
Error	5	16.75	

++ P=0.01

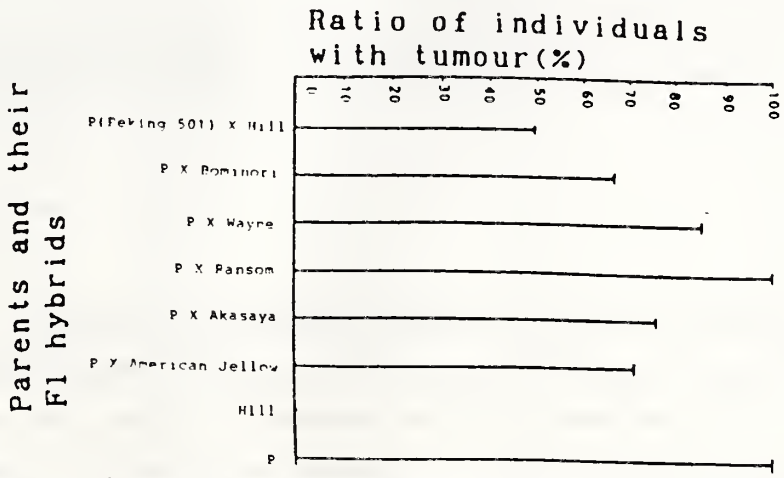


Fig 1 The Ratio of Individuals with Tumors in F1 Hybrids

The relationship between parents and their F1 hybrids was considered. On trait percentage of tumor induction per plant, a positive correlation between parents and their F1 hybrids existed, R=0.90 (P=0.01) (Fig. 2), displaying that is reliable to prejudge F1 genotypes by their parent genotypes or phenotypes. Otherwise on trait average size of crown gall per plant, no significant correlation was obtained between parents and F1 hybrids (Fig. 3).

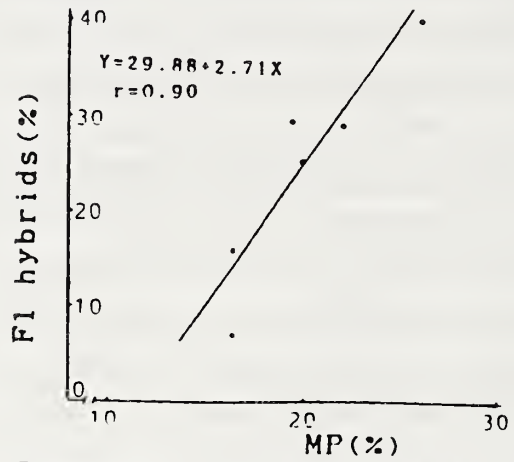


Fig 2. Regression btween parents and F1 hybrids on percent tumorigenesis per plant

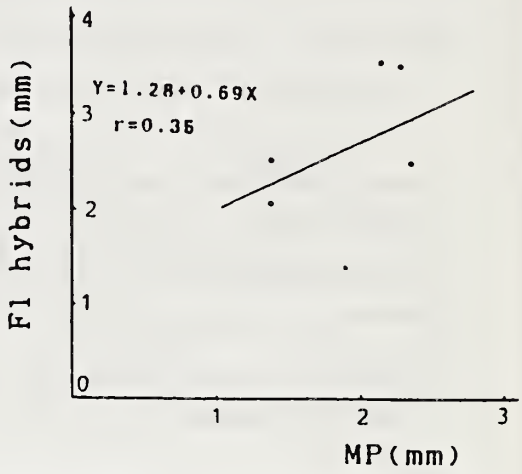


Fig 3. Regression btween parents and F1 hybrids on average size of tumor per plant

Furthermore, combining ability of parents and heritability of the two traits were calculated. Table 2 showed that percentage of tumor induction was mainly modified by additive gene action and possessed a higher narrow-sense heritability than average size of crown gall. In contrast, average size of crown gall had a poor variance of general combining ability, substituted with a rich variance of special combining ability, which revealed there was not much possibility to foresee F1 expression of crown gall size by their parent phenotypes or genotypes. Especially, expected genetic component might not reliably be inherited to next generations due to gene dominance and epistasis action in this trait.

Table 2. The analysis of combining ability and heritability in F1 hybrids

	%			
Characteristic	Vgc	Vsc	Hb	Hn
Percentage of tumor induction per plant (%)	73.64	26.36	70.20	55.11
Average size of crown gall (mm)	53.48	46.52	68.72	44.50

To determine the competent combinations from which superlines highly susceptible to A208 could easily be selected, appraisement was conducted. According to Table 3, Peking 501 X Ransom appeared to be the optimal combination for the development of A208/susceptibility lines, next were Peking 501 X Akasaya 1 and Peking 501 X Wayne.

Table 3. Combination appraisement

Combinations	Percentage of tumor induction		Average size of crown gall		Combination Appraisement
	Deviation	Range	Deviation	Range	
Pek X Hill	-68.68	(1) x 2*	1.93	(4) x 1*	6
Pek X Bon	-37.33	(2) x 2	-17.76	(2) x 1	6
Pek X Way	29.38	(5) x 2	-2.31	(3) x 1	13
Pek X Ran	53.61	(6) x 2	34.74	(5) x 1	17
Pek X AKA	18.37	(4) x 2	37.06	(6) x 1	14
Pek X A.Jel	4.64	(3) x 2	-53.66	(1) x 1	7

*weight

References:

Di., T. Kudirka, et. al. 1986. Can. J. Genet. Cytol. 128:809-817.
Owens, Lowell, D. et al. 1988. Plant Physiol. 88:570-573.
Byrne, M.C. et al. 1987. Plant Cell. Tissue and Organ Culture 8:3-15.
Otten, L. et al. 1978. Biochem. Biophys ACTA 527:497-500.

Lee Wenbin
T. Komatsuda

NORTHEAST AGRICULTURAL UNIV.
China
and
NATIONAL INSTITUTE OF AGROBIOLOGICAL RESOURCES
Japan

Binary vector mediated transformation of soybean

Introduction: Several methodologies for transformation of soybean have been described. Using agrobacterium nondisarmed vectors (including Ti and Ri plasmids), no transformed plant was obtained due to a barrier of plant regeneration from tumor callus (2). Transient transformants of Kanamycin-resistance were obtained by using virulent A. tumefaciens strains carrying a binary vector with a chimeric neo gene(4). Transformed plants, integrated with a resistant gene to herbicide glyphosate or kanamycin, were regenerated via organogenesis and consistently inherited(3). A direct gene transfer to protoplast was achieved through electroporation and DNA coated gold particles techniques (6). An expression of hygromycin phosphotransferase gene has been reported from transformed shoots via protoplast culture(5).

Despite these achievements, to date, transformation frequency of soybean is still low relative to that obtained in alternative plant hosts such as tobacco and petunia. The limitation partially was due to a lack of an effective transformation system. At an attempt to improve transformation efficiency, present experiment was undertaken to compare the effect of gene transformation between two regeneration systems, embryogenesis and organogenesis, with different disarmed vectors and soybean genotypes.

Materials and Methods:

Embryogenesis procedure

Plant of soybean genotypes 'Peking 501', 'American Jellow', 'Kou 502' (Masshokutou), and 'Bominori' were grown in greenhouse. Embryos were excised from immature seeds and incubated in petri dishes with 30 ml of gelrite-solidified embryogenesis medium containing MSB+10g/L sucrose+10mg/L NAA. The explants were then inoculated with an overnight suspension of EHA101/PSAORI221 or LBA4404/PTRA415 by dripping around the wounding area. PSAORI221 is a binary Ti plasmid that contains the beta-glucuronidase (GUS) gene drived by the CaMV35S promoter and neomycin phosphotransferase gene.

The PTRA415 was harbored with a tobacco PR1a protein gene which was an inducible promoter by stress or chemicals.

After one day's co-cultivation, explants were transmitted onto the same medium, with the exception of the addition of 250mg/L carbenicilin. The selection of kanamycin resistance was conducted after ten days.

Organogenesis procedure

Soybean genotypes Peking 501 and Bominori were used to gene transformation via organogenesis. Seeds were germinated in petri dishes containing MS medium supplemented with 1mg/L benzyladenine (BA), sucrose 20g/L and agar 8g/L (Disco agar) for two days. Explants were prepared by slicing the embryonic axis into two halves while still attached to the cotyledons. The epicotyl and hypocotyl were removed 1mm from the cotyledonary node.

Explants were immersed in the overnight suspension of EHA101/PSAORI221 and LBA4404/PTRA 415 for four min and were then placed abaxial side down on shoot induction medium containing MS salts (except the inorganic nitrogen, which was replaced by 1g/L L-glutamine), MS vitamins, 1 mg/L BA, 0.7 mg/L gibberellic acid (GA), 10 g/L sucrose and 8g/L Difco agar. Forty-eight hrs later, explants were moved to new induction medium supplemented with 250mg/L carbenicilin and 50 mg/L kanamycin (sulfate). Fifteen days later, regenerated shoots were excised and transferred to root induction medium.

B-Glucuronidase (GUS) assays

The GUS activity was determined in regenerated plantlets by both histochemical reaction and fluorometric assays according to Jefferson (1). Histochemical reaction was performed by incubating tissues in 100 µl of 5-Bromo-4-chloro-3-indolyl glucuronide (X-gluc) at 37°C until development of blue color (48 hrs). Fluorometric assays were carried out to quantify GUS activity in extracts prepared from young shoots. GUS activity was expressed as 4-MU pmoles produced in 30 min at 37°C per g fresh tissue.

Results: Sensitivity of seedling explants to kanamycin was estimated prior to actual transformation experiments in order to determine the effective concentration for selection. In the absence of kanamycin, the seedling explants regenerated normally and produced multiple shoots on the meristematic ring. The capacity of shoot regeneration was inhibited even at 50mg/L of kanamycin. Higher kanamycin concentrations were very toxic to seedling explants and caused immediate yellowing (Table 1). Hence, concentration 50mg/L was initially used for selection of transformed shoots.

Meanwhile, the sensitivity of immature cotyledons to carbenicilin was also estimated to protect safety of embryogenesis. A considerable impact of carbenicilin was observed in embryogenesis proceeding. Concentration increment from 0 to 300 mg/L stimulated embryogenesis and increased average number of normal embryos. Especially, the average weight of somatic embryos and average length of embryos were unexpectedly enhanced, and morphologically, cotyledonary callus grew larger than in normal conditions (Table 2). Hence, 250 to 300 mg/L carbenicilin was the effective concentration. Higher concentration resulted in the reducing of embryogenesis efficiency.

Table 1. Effect of different concentration of kanamycin on organogenesis

Kanamycin Concentration	Total explants	Percentage of explant with shoots	Shoot numbers per explant
0	36	88.89	17.25
50	36	100	7.33
100	36	66.67	5
200	36	33.33	4
500	36	0	0

All transformation attempts were summarized in Table 3 and Table 4. Immature embryo co-cultivation with LBA4404/PTRA415 totally produced 748 somatic embryos; of them no transformant was recovered. A similar result was obtained from seedling explant co-cultivated with LBA4404/PTRA415, indicating that LBA4404 was seemingly avirulent or weakly virulent to soybean genotypes. In contrast to LBA4404/PTRA415, 0 to 12.23% transformants were selected from different genotypes via two regeneration proceeding by co-cultivation with strain EHA101/PSAORI221. GUS histochemical assay and fluorometric assay displayed a positive activity of GUS gene. In present experiment, organogenesis proceeding appeared to be higher capacity of gene transformation than embryogenesis proceeding.

Table 2. Effect of Cb concentration on embryogenesis

Cb Concentrations (μg/ml)	Frequency of Embryogenesis	Normal Embryos	Weight of Embryos (mg)	Length of Embryos (mm)
0	85.18	5.7	0.61±0.13	0.84±0.14
50	100	5.8	1.75±0.10	0.89±0.15
100	100	6.0	1.80±0.25	0.91±0.04
250	100	6.7	4.06±0.12	1.71±0.19
300	100	7.17	4.58±0.35	1.89±0.17
500	83.33	4.8	0.32±0.05	0.81±0.09
700	76.67	4.78	0.22±0.02	0.55±0.02

Table 3. Transformants from embryogenesis proceeding

	EHA101/PSAORI221		LBA4404/PTRA415	
Genotypes	Total Embryos	Percent Transformation (%)	Total Embryos	Percent Transformation (%)
Peking 501	298	5.36	150	0.00
Kou 502	410	2.43	200	0.00
A.Jellow	203	0.98	168	0.00
Bonminori	178	0.00	230	0.00

Table 4. Transformants from organogenesis proceeding

	EHA101/PSAORI221		LBA4404/PTRA415	
Genotypes	Total Shoots	Percent Transformation (%)	Total Shoots	Percent Transformation (%)
Peking 501	188	12.23	105	0
Monminori	150	4.00	148	0

References:

Jefferson, R.A. et al. 1987. EMBO J. 6:3901-3907.
Bryne, Michael C. et al. 1987. Plant Cell, Tissue and Organ Culture. 8:3-15.
Maud, A. W. Hinchee et al. 1986. Bio/Technology. 6:915-923.
Christou, Paul et al. 1991. Soybean Genet. Newsl. 18:201.
Christou,Paul et al. 1987. Proc. Natl. Acad. Sci. USA 84:3960-3966.
Sarwan, K.D. et al. 1991. Plant Cell Reports. 10:106-110.

Lee Wenbin
T. Komatsuda

MENDELEUM
Dept. of Genetics and Plant Breeding
Horticultural Faculty
Univ. of Agriculture, Brno
691 44 Lednice na Moravè
Czech Republic

Screening of enzyme systems

Introduction: The aim of this paper was screening, especially of enzyme systems of the group of parental soybean genotypes and their hybrid progenies. Obtained information was expected to be used for identification of successfulness of hybridization of such species which can not easily be hybridized and self-pollinated, such as soybean, for certification of final breeding products and for opening of research of potential linkage (association) between biochemical (enzyme) traits and quantitative traits, which are responsible for manifestation of economically significant traits and plant properties.

Preparation for this aim was taken from the papers given by Wagner, MacDonald, 1981; Goodman, Stuber, 1980, 1983; Cardy, Kennenberg, 1983; Vallejos, Tanksley, 1983; Cardy, Beversdorf, 1984; Dong, Kiang, 1987; Wendell, Weeden, 1989; Delorme, Skorupska, 1992; and Kadlec et al., 1994.

Materials and Methods: Starting experimental material were seeds of eight parental genotypes from germplasm in Mendeleum and their nine hybrid combinations at the standard of generations F_2 and F_3, respectively. The following 16 enzyme systems were used for biochemical analyses: ACP - acid phosphatase, ALD - aldolase, ADH - alcoholdehydrogenase, AMY - amylase, DIA - diaphorase, EST - estarase, IDH - NADP - active isocitratedehydrogenase, LAP - leucineaminopeptidase, Ep - peroxidase, PGD - phosphogluconate dehydrogenase. PGI - phosphoglucose isomerase, SBTI - soybean trypsin inhibitor, URE - urease, SOD - superoxide dismutase, CBB - native proteins dyed.

Adapted electrophoretic system according to "Pharmacia ExelGel™ technical data sheet" was used for native conditions and for vertical electrophoresis according to stejskal (not published). Native electrophoretic discontinuous system according to Laemmli (1970) with 7.5% polyacrylamide gel was applied in the case of analysis of total proteins of seeds.

Electrophoresis was carried out in native discontinuous system of polyacrylamide gel in structure of 7.5% polyacrylamide, 0.12 M Tris, 0.12 M acetate, pH 6.4 gel proportion 8.3 x 5.5 x 0.075 cm.

Focusing gel was formed by 5% polyacrylamide and buffer 0.05 M Tris acetate, pH 6.0.

Electrode buffer system was formed by 0.075 Tris 0.075 M acetate, pH 6.4 in anode space and 0.02 M Tris + 0.2 M Tricin, pH 7.1 for cathode.

Seed proteins (enzymes) were extracted from a dry seed. The seed was relieved of testa and it was used for analysis of Ep activity. Cotyledons were homogenized to flour in a grinding mortar.

Flour extraction was carried out by 0.125 Tris - HCl by a buffer pH 6.8 with addition 10% of glycerol and 10 mM mercaptoethanol in proportion 100 mg of flour per 1 ml of buffer, 30 min. on the icy bath.

Centrifugation ran at 30000g - 4°C - 10 min. Clear supernatant was taken after centrifugation from under lipidic layer to new eppendorfs.

4μl of extract per pit, at the comb with 10 pits of the apparatus Midget was for the electrophoresis itself, which represents a limited amount of applied proteins which do not cause smudging of isoenzymes in the gel.

Protein dyeing was carried out by the standard procedure, Coomassie Blue R 250, in some cases with an overlay correction, dyeing of isoenzyme group activities according to Vallejos (1983).

Peroxidase activity test of testa was carried out by moistening of testa of one soybean seed in 2 ml 0.05% benzidine (10 minutes) and by addition of hydrogen peroxide to concentration 0.03%. Velocity and intensity of blue dyeing of the solution was visually evaluated.

Results and Discussion: Hybrid character of seeds of studied parental combinations was corroborated on the basis of analyzed biochemical traits and, in relation to the whole purpose, it was on the basis of analysed biochemical traits and in relation to the whole purpose and it was on the basis of enzyme systems AMY, ACP, URE, SOD, DIA, PGM, and CBB proteins, except hybridization 020 x 061, which is correspondent to genotype 020 in all biochemical traits.

All analyzed genotypes can be reliably differentiated except the given genotype couple 020 and 061.

In the given genotype group enzyme systems, AMY, ACP, URE, SOD, DIA, and PGM can be considered to be excellent and totally unambiguous biochemical markers, observed in the system PAGE -seed.

Hybrid character of enzyme markers AMY, PGM, URE was even proved. It was also in ACP in one case, but it will be necessary to explain it because it is an unusual phenomenon. An indication of hybrid character appeared in the case of CBB by SBTI systems, too.

Unlike literary resources IDH, ADH, and LAP were not corroborated as good enzyme systems and experiments with them will be repeated. The case of a low activity of the systems ALD and IDH can be solved with helping of leaves and by substitution of polyacrylamide gel by starch one in the case of IDH system.

Discovery of very interesting genotypes also contributed to the whole positive results of biochemical analyses, it was especially the form under number 059. Genome of the given genotype is not only a bearer of unique properties, but it has also an ability to transport these remarkable properties to progenies.

All given results are summarized and tabulated in a concentrated form, in pictures 1 and 2, where concrete enzyme systems and their forms are coordinated to concrete genotypes supplied by their control numbers. Possibility for certification of individual genotypes was opened in this way.

The way to analysis of quanititative traits in association with the given biochemical markers was opened by discovery of enzyme systems with a high activity and segregation, by discovery of unique genotypes.

Conclusion: During searching and investigation of biochemical markers 16 enzyme systems of the group of 17 genotypes were verified with the help of electrophoresis. From these systems ACP, AMY, PGM, URE, SOD, and DIA appeared to be very good from the point of view of a high activity and a segregation; EST, ALD, IDH and proteins CBB were also potentially good. On the contrary, Ep, SBTI and PGI did not show a suitable polymorhphism; ADH was hardly evaluating or LAP and PGD showed a very low activity.

The unique genotype which was able to transport its uniqe properties to its progeny was discovered in the group.

Obtained result allow verification of seed paternity in the earliest generations in the frame of given genotype group. They open the possibility of genotype certification and opening of the presumed linkage (association) research between marker and quantitative traits.

References:

Cardy, B.J., L.W. Kennenberg. 1982. Allozymic variability among maize inbred lines and hybrids: Applications for cultivar identification. Crop Sci., 22:1016-1020.

Cardy, B.J., W.D. Beversdorf. 1984. Identification of soybean cultivars using isoenzyme electrophoresis. Seed Sci. Technol. 12:943-954.

Delorme, R.M., H. Skorupska. 1992. New isoenzymes in soybeans. Soybean Genet. Newsl. 19:169-173.

Dong, J.Y.H., Y.Y. Kiang. 1987. Cultivar identification by isozyme analysis. Soybean Genet. Newsl. 14:189-226.

Goodman, M.M., C.W. Stuber. 1980. Genetic identification of lines and crosses isoenzyme electrophoresis. Proceedings of the Annual Corn and Sorghum Industry Research Conference. 35, 10, 31.

Goodman, M.M., C.W. Stuber. 1983. Isoenzymes of maize. In Isoenzymes in Plant Genetics and Breeding (eds. S.D. Tanksley and T.J. Orton). 1-33 Elsevier Scientific Publishing Co., Amsterdam, Netherlands.

Kadlec, M., J. Stejskal, J. Letal, M. Griga, J. Golias. 1994. Investigation of genetical markers in relation to quantitative traits. Agricultural University Brno, Mendeleum - Dept. of Genetics and Plant Breeding of Horticultural Faculty in Lednice, 21 pg, 28 tb, 20 fig.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Vallejos, C.E., S.D. Tanksley. 1983. Segregation of isoenzyme markers and sold tolerance in an interspecific backcross of tomato. Theor. Appl. Genet. 66:241-247.

Wagner, C.K. and M.B. McDonald, Jr. 1982. Rapid laboratory tests useful for differentiation of soybean (Glycine max) cultivars. Seed Sci and Technol. 10:431-449.

M. Kadlec
J. Stejskal
J. Letal
M. Griga
J. Golias

Figure 1 **DIAGRAMMATIC REPRESENTATION OF ELECTROPHORETIC BANDING PATTERNS FOR 11 ENZYMES OF SOYBEAN CULTIVARS**

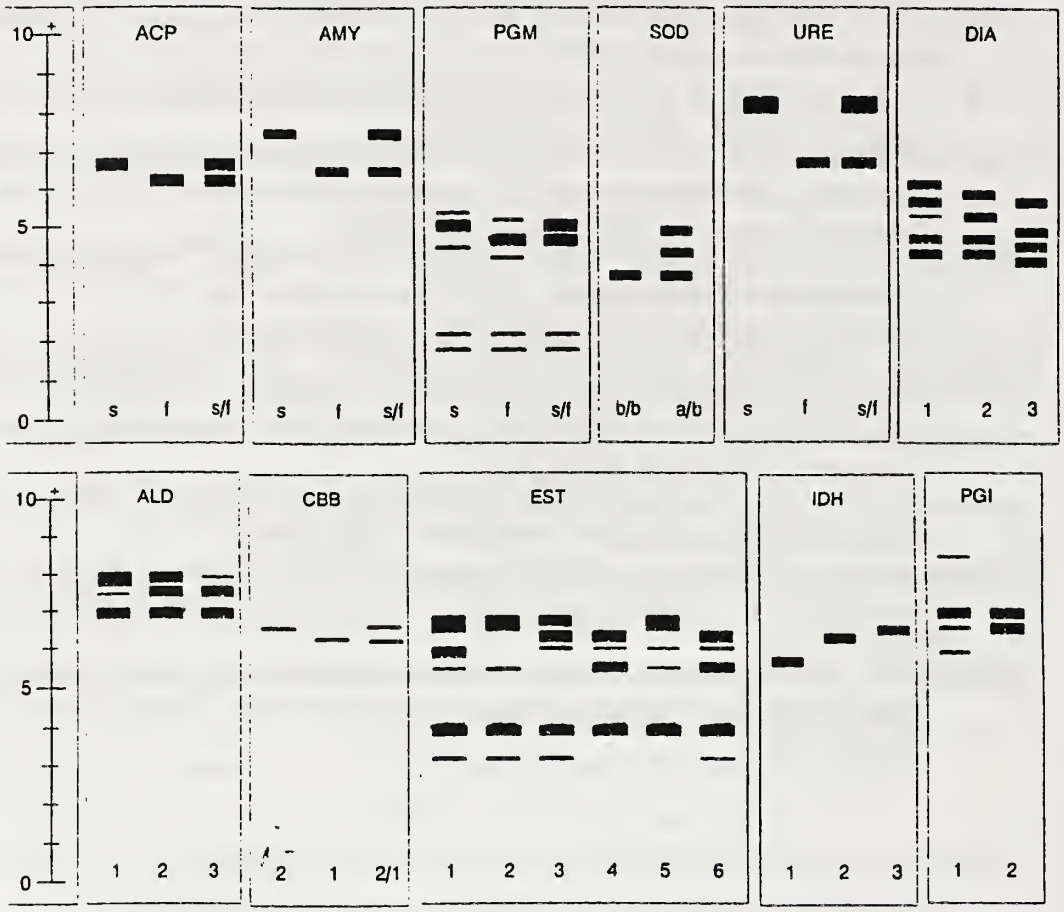


Figure 2 **RESULTS OF BIOCHEMICAL ANALYSIS OF SOYBEAN CULTIVARS**

Number of arrange	Number of cultivars in genobank	F_n	BIOCHEMICAL CHARACTERS											
			ACP	AMY	PGM	SOD	URE	DIA	ALD	CBB	EST	IDH	PGI	Ep
1.	059		s	s	s	b/b	s/vha	1	2	2	1	1	1	h
2.	020		f	f	f	a/b	f	1	3	1	2	2	1	h
3.	041		f	f	f	a/b	f	1	2	1	2	3	1	h
4.	076		s	f	s	a/b	f	1	1	1	1	1	1	h
5.	018		f	f	s	a/b	f	2	2	1	1	3	1	h
6.	077		f	f	f	a/b	f	1	1	1	3	2	1	h
7.	061		s	f	f	a/b	f	2	2	1	1	2	1	h
8.	055		f	f	s	a/b	s	1	1	1	4	1	1	l
9.	020x055	F_3	f	n.d.	f	a/b	s	1	1	1	6	1	2	h
10.	020x055	F_2	f	f	f/s	a/b	f	1	1	1	5	3	1	h
11.	020x061	F_2	f	f	f	a/b	f	1	3	1	2	2	1	h
12.	059x018	F_2	s	f/s	s	b/b	s	3	1	2	1	1	1	h
13.	059x077	F_2	s	f/s	f/s	a/b	f/s	2	2	2	2	1	1	h
14.	059x061	F_2	f/s	f	f/s	b/b	f/s	1	2	1/2	2	1	1	h
15.	041x076	F_2	f	f	f/s	a/b	f	3	2	1	2	2	1	h
16.	041x018	F_2	f	f	f	a/b	f	2	2	1	2	3	1	h
17.	041x061	F_2	f	f	f	a/b	f	3	2	1	2	2	1	h

Note:

1 až 6:	kind of isoenzym spectrum
f:	fast
s:	slow
f/s:	combining
a/b:	genotyp
b/b	genotyp
vha	very high activity
n.d.	don't detection
h	high activity
l	low activity

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Indore 452001
M.P. India

Pod-wall thickness as a selection criterion for shattering resistance in soybean

Introduction: Pod-shattering in soybean causes serious yield losses in tropical and sub-tropical countries. Varieties are differentially prone to pod-shattering and discerning their consistency for shattering-resistance may require several years of testing (Tiware and Bhatnagar, 1993). To provide suitable selection criteria and to probe into structural basis of resistance to shattering, pod-anatomy of soybean varieties displaying a wide range of shattering was carried out. The results obrained are contained in the present report.

Materials and Methods: Sixteen divergent soybean varieties were studied for pod-anatomical characteristics vis-a-vis pod-shattering using largely the methods of Tiwari and Murthy (1986) and Tiwari and Bhatnagar (1993). For assessing the effectiveness of pod-wall thickness as a selection criterion, further analysis was done using a larger sample of 35 varieties and strains.

Results and Discussion: A wide range of variability was observed for all the characters studied except for epicarp thickness which was excluded from analysis. Sclerenchymatous structures of pod, viz. bundle cap length and its thickness, were observed to be highly associated with shattering resistance when compared with epidermis and hypodermis thickness (Table 1). These sclerenchymatous characteristics were found to be associated with pod-wall thickness. Pods with thick walls contained more sclerenchyma and better resistance to pod-shattering when compared with the pods having relatively thin walls.

When varieties were categorized on the basis of shattering resistance and anatomical characters, the best non-overlapping ranges and discreet mean values were obtained for pod-wall thickness (Table 1). The association was further tested using a large sample. As expected, pod-shattering and pod-wall thickness exhibited a strong negative correlation ('r' = -0.779; significant at 0.01 P). Utility of pod-wall thickness was also confirmed by residual analysis. The regression equation for the sample was found to be: Pod-shattering (%) = 150 - (0.248 x podwall thickness in μ. Pod-wall thickness is easy to measure in comparison to the other complex anatomical characters associated with pod-shattering and, hence, could serve as a feasible selection criterion. For screening, it would be desirable to include confirmed check varieties like 'Bragg' (highly resistant), 'PK 472'

Table 1. Pod-anatomical characteristics of soybean varieties belonging to different categories of pod-shattering intensity

Group of varieties	Mean pod shattering per cent ±S.E.	Mean values in u ±S.E.						
		Pod wall thickness	Epidermis+ hypodermis thickness	Inner sclerenchyma thickness	Bundle cap length on		Bundle cap thickness at centre	
					Dorsal side	Ventral side	Dorsal side	Ventral side
1. *G. soja*, *G. soja* derivative	100.0±0.0	352.1±56.2	54.2±8.3	72.1± 4.2	458.3±10.4	380.2±36.5	109.4±15.6	72.9±10.4
2. NRC 1, Punjab 1	72.4±4.7	414.6± 2.0	60.9±2.6	84.4±13.5	802.1±31.2	598.9±78.1	119.8± 5.2	130.2±15.6
3. T-49, NRC 2, JS 80-21, MACS 13, Lee	42.5±4.6	458.7±11.2	64.2±1.4	86.7± 5.1	889.6±47.3	608.3±33.4	202.1±14.2	150.0±12.6
4. Hardee, MACS 58, PK 564, JS 75-46	21.5±2.9	487.0± 7.1	67.1±0.8	98.2± 6.0	1010.4±44.4	653.6±25.3	214.8± 8.8	174.5±18.7
5. NRC 3, PK 416, Bragg	7.1±3.2	537.5± 7.3	71.2±4.0	100.7± 7.7	1020.8±42.1	750.0±83.3	236.1±13.9	156.2±27.5

(moderately resistant) and 'JS 2' (highly vulnerable) for comparison. Strains and segregants surpassing the moderately resistant checks could be selected.

Conclusion: Pod-wall thickness was found to be a reliable selection criterion for shattering-resistance in soybean. The character is easy to measure. Pods with thick walls possessed increased sclerenchyma and high resistance to pod-shattering

References:

Tiwari, S.P. and T.G.K. Murthy. 1986. A rapid method for plant anatomy using fluorescence microscopy. Current Sci. 55:362-263.

Tiwari, S.P. and P.S. Bhatnagar. 1993. Consistent resistance for pod-shattering in soybean varieties. Indian J. Agric. Sci. 63:173-174.

V.S. Bhatia
S.P. Tiwari

NATIONAL RESEARCH CENTER FOR SOYBEAN
Khandwa Road, Indore India

Genotypic compatibility for yield in variety blends of soybean

In India, farmers occasionally sow varietal mixtures of soybean, but no systematic study on the yield performance of soybean variety blend has been conducted. In this study, performance of variety blends involving six popular varieties in equal proportion (1:1) is reported.

Materials and Methods: Six varieties viz. JS 71-05, JS 80-21, MACS 58, PK 416, PK 472 and Pusa 16 were mixed in equal proportion to make 15 possible variety blends in 1:1 ratio. These 15 blends, along with the six pure varieties, were grown during rainy seasons in two consecutive years (1992-1993). Each trial was laid out in randomized block design with three replications. Individual plot size was 8m x 1.35 m with the spacing of 45 cm between rows and 5 cm between plants. Yield data were recorded and expected yield were calculated using the mean yield data of two seasons.

Results and Discussion: Blends were evaluated for their seed yield compared to that of the varieties grown in pure stand. Differences in yield among blends and varieties were highly significant, but none of the blends proved significantly higher yielding than the highest yielding component (Table 1). However, three blends viz. JS 71-05 + PK 472, JS 71-05 + PK 416 and PK 472 + PK 416 were equivalent to their highest yielding component. Earlier studies report that blends of soybean in general yield slightly better than the appropriately weighted component, but no better than the high yielding component (Probst., 1957, Mumaw and Webber, 1957, Brim and Schutz, 1968).

Assuming that the yield of components in pure stand will add up to give the yield of a blend, the expected yield of an equal proportion (1:1) blend will be the average of the yields of the two varieties comprising that blend. A deviation in the observed yield from the expected yield will indicate the existence of genotypic competition effects. A net gain in yield will indicate overcompensation while loss in yield is undercompensation. Five blends showed overcompensation varying between 3.58% and 9.10%, while four blends showed undercompensation varying between 5.10% and 10%. The present study indicates that since the blends do not give significant yield advantage over the best variety, they may be recommended for advantages other than yield. Further studies with blending of more diverse

varieties in varying proportions may help to explore favorable genotypic compatibility among Indian soybean varieties.

References:

Brim, C.A. and W.M. Schutz. 1968. Inter-genotypic competition in soybeans. II. Predicted and observed performance of multiline mixtures. Crop Sci. 8:735-739.

Mumaw, C.R. and C.R. Weber. 1957. Competition and natural selection in soybean varietal composites. Agron. J. 49:154-160.

Probst, A.H. 1957. Performance of variety blends in soybeans. Agron. J. 49:148-150.

P.S. Bhatnagar
P.G. Karmakar
Prabhakar
Vipin Kumar

Table 1. Mean seed yield of the varieties in pure stand and observed and expected mean yields of 2-component (1:1) variety blends

Variety/Blend	Observed yield (q/ha)	Expected yield (q/ha)	Increase or decrease over expected yield (%)	Yield of better parent (q/ha)
JS 71-05	26.05			
+ PK 472	26.58	24.81	+ 7.13	26.05
+ PK 416	26.52	26.20	+ 1.22	26.35
+ Pusa 16	21.38	23.05	- 7.25	26.05
+ JS 80-21	22.59	21.81	+ 3.58	26.05
+ MACS 58	21.15	21.10	+ 0.24	26.05
PK 472	23.56			
+ PK 416	25.62	24.96	+ 2.64	26.35
+ Pusa 16	21.36	21.80	- 2.02	23.56
+ JS 80-21	22.43	20.56	+ 9.10	23.56
+ MACS 58	20.00	19.85	+ 0.76	23.56
PK 416	26.35			
+ Pusa 16	23.92	23.20	+ 3.10	26.35
+ JS 80-21	20.84	21.96	- 5.10	26.35
+ MACS 58	19.75	21.25	- 7.06	26.35
Pusa 16	20.04			
+ JS 80-21	16.92	18.80	- 10.00	20.04
+ MACS 58	19.68	18.09	+ 8.79	20.04
JS 80-21	17.56			
+ MACS 58	17.82	16.85	+ 5.76	17.56
MACS 58	16.14			
Mean	21.74			
CD (5%)	3.34			

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Indore 452001, M.P.
INDIA

Seed-filling duration and height of insertion of the lowest pod in Indian soybean varieties

Introduction: Seed-filling duration is reported to have a positive association with seed yield in soybean (Dunphy et al. 1979; Nelson, 1986). Besides high yield, it will be desirable to have sufficient gap between the ground level and the point of insertion of the lowest pod for efficient mechanical harvesting of soybean (Krausse, 1989). Hence, Indian soybean varieties were screened for these two desirable characters and the results are reported.

Materials and methods: Forty-seven soybean varieties were grown during rainy season of 1993 at Indore (22° 41' N latitude, 75° 52' longitude and 540 meters altitude). Seed-filling duration was determined following Reicosky et al, (1982). Data on lowest pod insertion height, mature plant height and related phenological characters were also recorded.

Results and discussion: The length of seed-filling duration among the varieties varied from 26.2 to 44.4 days (Table 1). The varieties were classified into four categories based on their seed-filling durations. Varieties having shortest (category I) to longest (category IV) seed-filling durations are listed below.

Category I : Co 1, Punjab 1, Improved Pelican, Kalitur, MACS 57, T 49

Category II : Alankar, Durga, Gaurav, Gujarat Soy 1, Gujarat Soy 2, Hardee, Lee, JS 76-205, JS 335, MACS 13, MACS 58, MACS 124, Monetta, NRC 2, KHSb 2, PK 471, PK 472, Pusa 37.

Category III : Ankur, Birsa Soy 1, Bragg, JS 2, JS 71-05, JS 75-46, JS 80-21, Kalitur Sel. (E), PK 308, PK 327, PK 416, PK 564, Pusa 16, Shivalik, Shilajeet, VLS 1, VLS 2.

Category IV : PK 262, Pusa 20, Pusa 22, Pusa 24, Pusa 40, SL 96.

Seed-filling duration showed strong positive association with reproductive period, seed weight and ield per plant (Table 2). It showed a negative association with days to flowering and pod-initiation and establishment. Varieties with long seed-filling duration generally showed long reproductive period, early flowering and a short pod-initiation and establishment.

Varieties differed considerably for both lowest pod insertion height (mean 16.31 cm; range 10.15 to 24.85 cm) and plant height (mean 74.40; range 36.65 to 111.10 cm). The lowest pod insertion height showed a strong positive association ('r' = 0.58; significant at 0.01 P) with plant height. Varieties were classified into six plant height groups and within each group according to their lowest pod insertion heights (Table 3). Varieties viz. MACS 58, Durga, Punjab 1, Shivalik, Kalitur and Pusa 40 possessed high insertion point of the lowest pod and were found to be suitable for mechanical harvesting with reapers and combines. Varieties viz. Pusa 40 and PK 262 had a desirable combination of long seed-filling duration and high insertion point of the lowest pod.

Conclusion: Several Indian soybean varieties with high insertion point of the lowest pod were identified to be suitable for mechanical harvesting. Height of insertion of the lowest pod was found to have a strong positive association with plant height. Suitability for mechanical harvesting could, hence, be achieved by selecting medium to tall varieties/ segregants in the breeding programmes. Two varieties viz. Pusa 40 and PK 262 had a desirable combination of high insertion point of the lowest pod and long seed-filling duration, a desirable character associated with high yield.

Table 1 .Seed-filling duration and related characteristics of soybean varieties of India.

Category	No.of varieties	Seed fill dura (days)	Reproductive period (days)	Pod initiation & estb. (days)	Days to flower	Days to maturity	Unit seed wt. (mg)	Yield per plant (g)
I	6	28.2	49.3	21.1	54.2	103.5	89	5.9
II	18	32.6	50.4	17.9	49.9	100.3	116	7.6
III	17	39.5	53.6	16.6	43.8	97.4	145	10.2
IV	6	41.9	57.2	15.3	44.8	102.0	142	9.2
Mean	--	34.9	52.3	17.4	47.6	99.9	126	8.5
S.E.	--	0.62	0.53	0.45	0.83	0.75	4.1	0.38

Table 2. Correlation coefficients for seed-filling duration and ralated characters in soybean.

Characters	Reproductive period	Pod initiation & estblishment	Days to flower	Days to maturity	Seed weight	Yield per plant
Seed filling duration	0.71**	-0.52**	-0.57**	-0.12	0.65**	0.50
Reproductive period		0.19	-0.45**	0.21	0.47**	0.40**
Pod initiation & establishment			0.24	0.40**	0.39**	-0.24
Days to flowering				0.78**	-0.75**	-0.59**
Days to maturity					-0.49**	-0.37*
Seed weight						0.58**

* = Significant at 0.05 P; ** = Significant at 0.01 P

Table 3. Two-way classification of soybean varieties based on lowest pod insertion height and plant height

	Lowest pod insertion height (cm)				
Plant height (cm)	10.0-13.0	13.1-16.0	16.1-19.0	19.1-22.0	22.1-25.0
35.0-48.0	JS 71-05	JS2, Shilajeet			
48.1-61.0	Birsa Soy 1, SL 96, PK 327	JS 335, Monetta, NRC 2, VLS 1&2, Pusa 20, PK 564	Bragg, PK472		
61.1 - 74.0		Pusa 37, Pusa 16	Alankar, MACS 13, PK 262, PK 471, PK 416, PK 308	Shivalik	
74.1 - 87.0	KHSb 2	Ankur, Guj. Soy 1, Pusa 24	Guarav, JS 76-205, MACS 57	Durga, Punjab 1	
87.1-100.0	Hardee, JS 75-46, Pusa 22	Guj, Soy 2, Imp. Pelican, Lee, MACS 124, JS 80-21, Kalitur Sel (E)	T49		
100.1 - 115.0			Co 1, Pusa 40, Kalitur		MACS 58

References:

Dunphy, E.J., J.J. Hanway and D.E. Green. 1979. Soybean yields in relation to days between specific developmental stages. Agron. J. 71: 917-920.

Krausse, G.W. 1989. Early ripening, productive soybean mutant variety suitable for combine harvesting. Mutation Breed. Newsl. 34: 3-4.

Nelson, R.L. 1986. Defining the seed filling period in soybeans to predict yield. Crop Sci. 26:132-135.
Reicosky, D.A., J.H. Orf and C.Poneleit. 1982. Soybean germplasm evaluation for length of the seed filling period. Crop Sci. 22:319-322.

P.G. Karmakar
S.P. Tiwari
Vipin Kumar

MAHATMA PHULE AGRICULTURAL UNIVERSITY
Agricultural Botany Section, College of Agriculture
Kolhapur - 416004 (M.S.) INDIA

Effects of Different Doses of Gamma Irradiation on Germination and Survival of Soybean

Introduction: Soybean [Glycine max (L.) Merr.] is an important pulse as well as seed crop as it contains high (43.20%) quality protein and about 20% oil. Its nodules also fix atmospheric nitrogen symbiotically. In view of the current edible oil crisis in our country, soybean crop has acquired obvious importance. The low average yield of soybean in India is the consequence of unavailability of high-yielding, biologically efficient, and input responsive varieties suitable for different cropping systems under varied agroclimatic situations. The present study was, therefore, undertaken to obtain information on the effect of gamma irradiation in inducing variability for different economic characteristics of soybean.

Materials and Methods: The five lots, each of 400 selfed, bold, uniformly dried seeds with 12% moisture, of five soybean varieties were exposed to different doses of gamma rays as per the details presented in Table 1. The treated seeds were sown in the field in a split plot design with three replications during summer, 1992. Germination count was recorded ten days after sowing (DAS). The number of seedlings that survived was recorded at the 21st, 28th, and 35th days. The survival percent was calculated.

Results and Discussion: The ANOVA presented in Table 1 indicated that there were significant differences in germination and survival percentage between varieties as well as doses and their interactions; Seedling survival at 21st, 28th, 35th days, and also at maturity

Germination:

1) Effect of varieties: There were non significant differences among the unirradiated (control) varieties for seed germination. However, the varieties showed highly significant sensitivity to the irradiation resulting in significant reduction in seed germination. Variety SOY-1 (49.46%) and SOY-4 (49.66%) were found most sensitive, whereas SOY-2 was tolerant (76.60%), followed by SOY-5 (73.80%) and SOY-3 (56.26%).

2) Effect of doses: There were non significant differences, in means of different doses or between the control and treated varieties, for germination percentage.

Seedling survival:

1) Effects of varieties: Seedling survival recorded after 21 days did not show any significant differences amongst unirradiated varieties. From the means of three observations, variety SOY-4 appeared to be the most significantly sensitive to gamma rays. Due to injury caused by gamma rays, only 38.24% plants survived as opposed to 67.10% in the control group and 41.55% in variety SOY-1, 49.15% in SOY-3, and 53.08% in SOY-2, which showed a large reduction, though not significant, for seedling survival. Surprisingly, variety SOY-5 was most resistant, in which highest survival (72.20%) was observed out of 73.99% germinated seeds.

2) Effects of doses: Average seedling survival over control varieties and at different intervals was 64.67, 48.48, 38.32, 51.06, 42.89, and 43.93% in the O (control), 10, 20, 30, 40, and 50 Kr., respectively. The seedling survival was found to be reduced significantly at 40 and 50 Kr. doses. In other doses, though not significantly, it was reduced to a greater extent.

The data on germination at tenth day indicated significant differences for survival between control and treated varieties. Varieties also differed significantly from each other for this character. At 21^{st} and 28^{th} days, varieties SOY-4 and SOY-1 showed maximum seedling mortality followed by SOY-3, SOY-2, and SOY-5, which were found to be most tolerant in ascending order. However, the trend of varietal sensitivity was changed at 28^{th} day indicating highest seedling mortality in SOY-2 (23.53%), followed by SOY-1 (30.33%), SOY-3 (42.93%) and SOY-4 (43.20%). There were significant differences for percentages of plant survival between treatments viz., unirradiated (control) and irradiated varieties. However, variety SOY-5 did not differ significantly for the percentage of plant survival.

Earlier workers Lee et al. (1968) reported higher germination after treatment of soybean seed with low doses of X-rays. The results reported in present studies were contradictory to Lee et al. (1968), which might be due to different material and sources of irradiation used in the present studies. The significant reduction in percent germination observed in present studies might be due to higher doses of gamma rays used. These findings are in agreement with Borejko (1970), who obtained reduced germination with chemical mutagenic treatment to the soybean seeds. Present studies indicated that increase in the dose rate increases the injury, thereby resulting in low germination as well as survival.

Regarding the effects of doses, highest reduction was observed in 40 Kr. (27.19%) followed by 50 Kr. (24.75%), 10 Kr. (21.04%), 30 Kr. (17.17%), and 20 Kr. (16.16%). Highest reduction in survival percent was in 50 Kr. (41.89%) followed by 40 Kr. (38.13%), 10 Kr. (33.59%), 20 Kr. (19.70%) and 30 Kr. (13.29%).

The effects of physical and chemical mutagen on gene and chromosomal mutations in the biological material could be measured quantitatively by degree of reduction in germination, seedling survival, growth and fertility (Gaul, 1970). According to Gaul, the high sensitivity observed in field germination and survival may be attributed to the injuries caused by mutagenic treatment. The affected seedling probably lacked vigor to come out of the soil surface. Georgiev and Topcieva (1970) reported critical dose 12 Kr. in some varieties and 16 Kr. in others on the basis of survival percentage of plants. In present studies, results obtained confirm the earlier results (Borejko, 1970).

References:

Borejko, A.M. 1970. Production of induced mutations in soybean. Genetika Moskva, 6(10): 167-169 (Russian).

Gaul, H. 1970. Mutagen effect observable in the first generation. I. Plant injury and lethality. II. Cytological effect. III. Sterility. Manual on mutation breeding IAEA, Vienna. Tech. Rept. Ser. No. 119, 85-99.

Georgiev, I. and A. Topcieva. 1970. A study of radio sensitivity of soybean to irradiation of seeds with gamma rays. Genetica Selekcia. 3:403-409 (Bulgarian).

Lee, B.H., E.R. Son, and D.K. Paik. 1968. Studies on the induced mutation breeding of soybean for bacterial blight resistance. J.Nucl.Sci. Seoul., 7:35-38.

S.S. Mehetre
C.R. Mahajan
P.M. Dhumal

Table 1. Analysis of variance of portioned sum of squares for varieties doses of gamma rays and varieties' "X" doses interactions

Source of variation	Degrees of freedom	MSS for Days after sowing				
		% Germ	% Survival			
		10 days	21 days	28 days	35 days	At maturity
Replications	2	1658.42	1006.55	2214.69	2558.44	2732.81
Varieties (v)	4	4187.84**	3527.78**	2340.94**	4419.18**	6046.81**
Error (a)	8	122.58	195.42	106.72	136.39	943.57
Doses (D)	5	175.46	198.48	546.49**	3719.82**	9219.74**
Interactions (V x D)	20	343.26**	315.203**	360.17**	799.996**	997.56*
Error	50	97.06	105.31	106.57	198.93	456.64

*,** = Significant at 5 & 1 % level, respectively.

Table 2. Mean germination (G) and survival (S) at 10 and 21, 28 and 35 days after sowing (DAS), respectively, in different varieties of soybean as influenced by different doses of gamma ray treatment (T) in comparison with control (C).

		Gamma Ray Doses Kγ.r				
Varieties	**Control**	**10**	**20**	**30**	**40**	**50**
	G--S	G--S	G--S	G--S	G--S	G--S
MPAU / ACK						
SOY-1	71.00-70.99	49.00-48.77	55.00-51.77	58.33-53.33	39.44-29.22	46.00-32.66
SOY-2	88.23-75.16	79.66-43.77	72.33-59.33	77.66-73.88	48.38-47.88	69.00-40.55
SOY-3	79.66-26.15	59.00-53.00	58.33-57.77	59.66-56.55	39.33-40.00	55.00-38.44
SOY-4	77.66-67.10	46.33-32.44	58.00-52.66	49.66-56.80	44.66-31.88	49.66-27.44
SOY-5	79.00-73.99	78.66-70.78	78.33-69.55	82.66-73.77	81.00-75.33	78.33-71.55
Mean	79.20-72.51	62.53-48.15	66.40-58.22	65.60-62.87	57.66-44.86	59.60-42.13
% Reduction over Control		21.04-33.59	16.16-19.70	17.17-13.29	27.19-38.13	24.75-41.89

MAHATMA PHULE AGRICULTURAL UNIVERSITY
Agricultural Botany Section, College of Agriculture
Kolhapur 416 004 (M.S.) INDIA

Induced Genetic Variability in the M_2 and M_3 Generations of Soybean

Introduction: Soybean is becoming an important crop in India and other developing countries. Besides oil (< 20%), it provides proteins (<40%) to ward off malnutrition and also to efficiently fix atmospheric nitrogen (Singh and SIngh, 1993), thus reducing the use of nitrogenous fertilizers.

The development of variety can be based on the magnitude of genetic variability of desired characters. It is rather difficult to obtain suffiecient cross seeds because of its very minute flowers as well as low percentage of successful crosses which are mainly dependent upon environment; personnel and (low) bearing of seeds/pod. Direct use of mutations is a very valuable approach to improve one or two easily identifiable characters in an otherwise well-adapted variety in the shortest amount of time. Desired mutation can be recovered in homozygous state in M_2 or M_3 generation as compared with the F_6 or F_7 generations in hybridization (Sigurbjornson, 1971). Present investigation was therefore attempted with a view to achieve targeted recombinations within the shortest possible time with the aid of gamma irradiation.

Materials and Methods: Seeds of uniform size and moisture content of four varieties viz., Monetta, MACS -57, MACS - 124, and MACS - 36 were treated with gamma ray at the rate of 10, 20, 30, 40 and 50 Kr. and sown in summer 1991. Observations were recorded for M_1 plant for seven different characters (Table 1-2) and harvested separately. From these, 197 plants showing full fertility and yield mean ± 2 SE were selected. Their M_2 progeny was raised along with their unirradiated control varieties in rainy season (Kharif, 1992) in completely randomized block design. All M_2 plants were considered for recording observations and selections were made according to the objectives as well as plants that were above the mean ± 2 SE for the respective characters under selection.

Progeny means of both M_2 and M_3 were used to estimate genotypic and phenotypic coefficients of variation (GCV and PCV); heritability (in broad sense) and genetic advance as per formulae suggested by Burton (1952); Singh and Chaudhari (1979) and Allard (1960).

Results and Discussion: Analysis of variance (Table 1) indicated significant difference among varieties, doses (except branches/plant in M_2 and pods/plant in M_3) and for variety dose interactions (except for plant height in M_3 days maturity, pods/plant and 100 grain weight in M_3) for all characters in both the generations (M_2 and M_3). Further, considerable variation ranging from 1.0% and 1.4% (days to 50% flowering) to 15.26% and 11.26% (grain yield/plant) in and M_2 and M_3, respectively, was observed. These results do not confirm earlier reports (Mhajan et al., 1993 and Jagtap and Mehetre, 1993).

In present investigations, only one character (i.e. plant height, 28.42, in M_2 and grain yield/plant in both M_2 and M_3, 28.14 and 36.19, respectively) showed moderate GCV, which indicated good scope for selection in M_2 and M_3. Whereas others had low GCV and hence limited scope for selection (Jagtap and Mehetre, 1993). In M_2 generation, plant height showed moderate GCV also had high heritability (89.40%) and grain yield/plant in both M_2 and M_3 generations showed moderate GCV accompanied by high heritability (85.90 and 90.80%). This indicates that these characters are highly heritable and less affected by the environment (Amaranatha et al., 1991). Further, Rajput (1987) suggested increased coefficients of variability provide oppportunity for selecting high yielding plants in M_2 generation. Moreover, Rajput and Sanwar (1988) also reported high heritability and genetic advance for grain yield/plant in M_2 generation of fast neutron irradiated soybean varieties. These results are confirmatory to the results obtained from studying irradiated as well as nonirradiated material by earlier workers.

High heritability values for days 50% flowering (M_2 and M_3) and maturity (M_3); plant height (M_2 and M_3); 100 grain wt. (M_3) and grain yield/plant (M_3) confirm the results reported from the studies of unirradiated material (Katariya and Sengupta, 1972; Amarnatha et al., 1993, and Jagtap and Mehetre, 1993).

In present studies high heritability was accompanied by high genetic advance for the characters viz., plant height (M_2 and M_3), grain yield/plant, branches/plant and pods/plant (M_2) and grain yield/plant (M_2 and M_3), which is very important to the breeder as selections based on these traits are highly effective, as well as amount of gain or superiority achieved can be maintained in subsequent generation (Jagtap and Mehetre, 1993) due to the prevalence of additive gene action (Naphade et al., 1972). Amaranath et. al., (1991) reported additive gene action for plant height and number of seeds/plant.

High heritability estimates does not necessarily mean an increasing genetic advance as reported earlier (Johnson et al., 1955) in soybean. According to Amarnath et al., (1991) days to 50% flowering and maturity involve nonadditive gene action.

As reported earlier, in present studies days to 50% flowering and maturity (Amarnath et al., 1991) and 100 grain weight (Amarnath et al., 1991 and Jagtap and Mehetre, 1993) showed high heritability and low genetic advance in M_2 and M_3 also indicated the involvement of nonadditive gene action.

Thus, it is concluded from the present studies that characters viz., plant height, yield/plant, pods/plant and branches/plant exhibited sufficient genetic variability in both M_2 and M_3 generations. Further, they were also accompanied by moderate to high heritability and genetic advance. Thus selection in M_2 and M_3 generation for these characters will provide superior progenies in M_3 and M_4 generations, respectively. For improving other characters viz., days to 50% flowering , maturity and 100 grain wt., some other breeding technique based on their high heritability estimates will have to be adopted as reported earlier (Jagtap and Mehetre, 1993).

The inconsistency in performance of different generations caused by genetic shift and breaking of gene linkages have been noted in chickpea (Bejiga et al., 1991). Inconsistent association among generations were also found in soybean (Weiss et al., 1947). Similar limitations are not revealed in present studies.

References:

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons. Inc. NY Lond. pp. 85-108.

Amarnath, K.C.N., S.R. Vishwanath and G. Shivshankar. 1991. Genotypes and phenotypic variability and heritability of some quantitative characters in soybean (Glycine max (L.) Merrill). Mysore J. Agric. Sci., 25:26-31.

Bejiga, G,. H.A. VanRheepen, C.A. Jagdish, and O. SIngh. 1991. Relationships among the F_2 to F_6 generations in chickpea (Cicer arietinum, L.) Indian J. Genet., 51(2):240-245.

Burton, G.W. 1953. Quantitative inheritance in grasses. Proc 6th Int. Grass Cong., 1:277-283,

Jagtap, D.R. and S.S. Mehetre. 1993.Genetic variability in some quantitative characters of soybean. Ann. Agric. Res., (in Press).

Johnson, H.W., H.F. Rovinson and R.E. Comstock. 1955. Estimates of genetic and environmental variability in Soybean. Agron. J., 47:314-318.

Katariya, A.S. and K. Sengupta. 1972. A study of the variability in Soybean [Glycine max (L.) Merr.]. Indian Agric. 16(2): 155-159.

Mahajan, C.R., P.A. Patil, S.S. Mehetre and R.D. Ghatge. 1993. Genotypic and phenotypic variability and heritability of some quantitative characters in soybean. Ann. Agric. Sci. (in Press).

Naphade, D.S., A.M. Mukewar and A.R. Kshirsagar. 1972. Morphological and genetic variability in some quantitative characters of soybean. PKV. Res. 1:80-83.

Rajput, M.A. 1987. Induced polygenic variance in soybean. Soybean Genetics Newsletter 14:117-119.

Rajput, M.A. and G. Sanwar. 1988. Induced Variability studies in Soybean. Soybean Genetics Newsletter 15:67-73.

Sigurbjornsoon, B. 1977. Introduction: Mutations in plant breeding programmes. In Manual on Mutation Breeding. IInd Ed. Tech Rep. Ser. No. 119, IAEA, Viena, pp. 1-6.

Singh, K.D. and B.D. Chaudhari. 1979. Variance and covariance analysis. Biometrical methods in Quantitative Genetics Analysis. Kalyani Pub.., New Delhi, p. 38-69.

Songh, K.D. and N.P. Singh. 1993. Uield and quality of Soybean [Glycine Max (L.) Merrill] as influenced by sol water at different stages of growth.

Weiss, M.G., C.R. Weber and R.R. Kalton. 1947. Early generation testing in soybean. J.Am.Soc. Agron. 39:791-811.

S.S. Mehetre
C.R. Mahajan
P.M. Dhumal
D.N. Hajare

Table No. 1 Estimate of mean, components of variance and heritability (broad sense) and expected genetic advance in respect of seven characters studied in M_2 and M_3 generations of Soybean.

Parameters	Days required for 50% flowering	Maturity	Plant height (cm)	Branches per plant	Pods per plant	100 grain weight (g)	Grain yield/plant (g)
M_2 generation							
Mean	56.05	101.11	35.13	4.24	64.88	11.56	13.02
Range (min.)	51.50	84.75	24.84	2.60	28.35	10.00	6.16
Range (max.)	60.25	106.25	66.50	5.61	85.70	13.00	19.44
C.V. %	1.00	1.21	9.59	9.80	10.87	55.48	15.26
Variance (G)	8.01	30.77	99.71	0.40	132.59	0.735	13.42
Variance (P)	8.33	32.27	111.06	0.571	191.44	1.137	17.392
Coefficient of variance (G)	5.05	5.49	28.42	14.92	16.72	7.42	28.14
Coefficient of variance (P)	5.15	5.62	30.00	17.82	21.33	9.22	32.03
Heritability %	96.20	95.40	89.40	69.9	69.3	64.600	77.2
Genetic advance % of mean	10.11	10.99	55.00	29.43	30.32	12.16	50.81

Table No. 1 (continued) Estimate of mean, components of variance and heritability (broad sense) and expected genetic advance in respect of seven characters studied in M_2 and M_3 generations of Soybean.

Parameters	Days required for 50% flowering	Maturity	Plant height (cm)	Branches per plant	Pods per plant	100 grain weight (g)	Grain yield / plant (g)
M_3 generation							
Mean	56.73	112.45	62.87	4.27	97.07	11.21	7.10
Range (min.)	52.45	108.04	50.35	3.06	75.10	9.56	2.44
Range (max.)	61.16	116.12	79.08	5.40	123.53	12.78	15.71
C. V. %	1.14	1.42	4.40	10.86	10.22	3.99	11.53
Variance (G)	7.86	5.66	46.46	0.195	79.10	0.889	6.605
Variance (P)	8.28	8.23	54.11	0.410	177.23	1.089	7.275
Coeffieient of variance (G)	4.94	2.12	10.84	10.34	9.16	8.41	36.19
Coefficient of variance (P)	5.07	2.56	11.70	15.00	13.72	9.31	37.99
Heritablility %	94.90	68.80	85.90	47.50	44.60	81.6	90.80
Genetic advance (% of mean)	9.82	3.57	20.49	14.52	12.43	3.66	70.43

*,** = Significant at 5 and 1% respectively.

Table No. 2 Analysis of variance of seven different characters in M_2 and M_3 generations of soybean.

Source	Degrees of freedom	Days required for 50% flower	Maturity	Plant height (cm)	Pods / plant	Brackets / plant	100 gram wt. (g.)	Yield / plant (g.)
M_2 generation								
Replication	1	3.219	0.094	8.105	748.743	0.022	3.00	18.611
Variances (V)	3	21.438**	77.115**	99.893**	159.620*	1.420**	2.741**	63.333**
Doses (D)	5	42.688**	27.031**	690.353**	58.431^{ns}	0.829**	3.846**	19.456**
V & D	15	6.631**	72.227**	73.094	430.494**	0.930**	1.039**	28.097**
Error	23	0.314	1.499	11.351	49.422	0.172	0.402	3.951
SE (V)		0.162	0.353	0.973	2.029	0.120	0.183	0.574
SE (D)		0.198	0.433	1.191	2.486	0.147	0.224	0.703
V & D		0.396	0.866	2.382	4.471	0.294	0.448	1.405
CD % (V)		0.642	1.403	3.861	5.938	0.476	0.727	2.278
CD % (D)		0.786	1.718	4.729	NS	0.583	0.890	2.290
V & D		1.573	3.436	9.458	19.734	1.165	1.312	5.580

*,** = Significant at 5 and 1 % respectively.

Table No. 2 (cont.) Analysis of variance of seven different characters in M_2 and M_3 generations of soybean.

Source	Degrees of freedom	Days required for 50% flower	Maturity	Plant height (cm)	Pods / plant	Brackets / plant	100 gram wt. (g.)	Yield / plant (g.)
M_3 generation								
Replications	1	1.969	5.188	82.52	72.44	0.006	0.008	0.475
Varieties (V)	3	26.135**	56.729**	78.51**	485.69**	1.570**	2.608**	49.042
Doses (D)	5	39.334**	13.700**	72.88*	372.86*	0.500^{ns}	1.820**	15.97**
V X D	15	6.403*	5.383^{ns}	114.22**	171.26^{ns}	0.447^{ns}	1.904*	6.15**
Error	23	0.422	2.560	7.65	98.39	0.215	0.200	0.67
SE ± (V)		0.188	0.462	0.799	2.863	0.134	0.129	0.236
SE ±(D)		0.230	0.566	0.978	3.507	0.164	0.158	0.239
V & D		0.459	1.131	1.956	7.014	0.328	0.316	0.579
CD at 1% (V)		0.744	1.834	3.171	11.367	0.52	0.513	0.938
CD at 1% (D)		0.912	2.246	3.883	10.26	ns	0.628	1.49
V X D		1.823	NS	7.766	NS	ns	1.256	2.298

NATIONAL RESEARCH CENTER FOR SOYBEAN
Khandwa Road
Indore India 452001

Evaluation of induced mutants of soybean for stem fly resistance and yield

Introduction: Induced mutagenesis in soybean is reported to be of utility in breeding for enhanced yield, desired seed coat color, flower color, high oil and protein contents, etc. (Bhatnagar et al. 1989; Bhatnagar and Tiwari, 1991; Ozbek et al., 1991). Induced mutations for insect resistance in soybean have not been reported. Insect pests of soybean, being one of the principle constraints in harnessing genetic yield potential in tropical countries like India, are of considerable importance. Accordingly, mutant progenies emanating from the study on defect rectification of soybean varieties by induced mutagenesis, were evaluated for resistance against [Melanagromyza soja (Zehnt.), Agromyzidae: Dipteral] and grain yield.

Materials and Methods: Thirteen mutant progenies of "PK 472" and seven of "Bragg", selected in M6 following irradiation of seeds to gamma rays (15, 20 and 25 Kr) with and without UV rays, were evaluated in rainy seasons of 1992 and 1993 for resistance to stem fly and yield. Experiments were planted in randomized block design with three replications in individual plots of 4.05 sq m area. Plant height and stem tunnelling, taken at 60 days after germination (DAG) and the seed yield at harvest, were compared with respective unirradiated control. All the mutants and controls were rated for relative susceptibility to stem fly on the basis of percent stem tunnelling, according to the procedure followed under All India Co-ordinated Research Project on Soybean (AICRPS) (Anon., 1992) as given below:

Rating	Criteria
Highly Resistant (HR)	Values between O and X - Critical differences (CD) at P=0.01
Resistant (R)	Values between HR and X - CD at P=0.05
Moderately Resistant (MR)	Values between R and X
Low Resistance (LR)	Values between X and X + CD at P = 0.05
Susceptible (S)	Values between LR and X + CD at P = 0.01
Highly Susceptible (HS)	Values more than S

Results and Discussion: Among the mutants of "PK 472", line 20Kr+UV '72-12' recorded significantly less stem tunnelling (23.12%) and higher grain yield (3979 kg/ha) than its control (Table 1). Mutant progeny 20Kr+UV '82-16' recorded maximum stem tunnelling (34.76%), however, it showed marked superiority over its control with respect to yield (3858 kg/ha), thus exhibiting high level of tolerance to damage by stem fly. Similarly, other mutants viz. 15Kr+UV '31', 20Kr+UV '82-7' and 25Kr+UV '30', though having stem tunnelling at par with its control, were significantly superior in yield. All the four mutant progenies mentioned above mature 1 to 3 days earlier than the parent.

In the case of the variety 'Bragg', which is highly susceptible to stem fly, all the selected mutant progenies recorded significantly less stem tunnelling (Table 2). Minimum stem tunnelling (23.31%) was observed in mutant progeny 20Kr '14-3', which was rated as resistant. Mutant progeny 20Kr+UV '95-10' yielded highest (3986 kg/ha). This mutant has purple flowers and matures at least seven days earlier. Earliness is a desirable character, particularly in rainfed soybean growing areas where subsequent crop is taken on residual soil moisture.

It is apparent from the two years study that radiation induced mutagenesis can bring about some desirable changes in the plants, which ultimately govern resistance to stem fly. There is need to study deviation in anatomical and biochemical characters of mutants from parents.

References:

Anonymous. 1992. Proceedings of 23rd Annual Workshop of All India Coordinated Research Project on Soybean. UAS, Bangalore, Karnataka. May 7-9, 1992. NRC for Soybean, Indore, pp.48-53.

Bhatnagar, P.S., Prabhakar and S.P. Tiwari. 1989. Improvement of soybean variety 'Bragg' through mutagenesis. Mutat. Breeding Newsl. 33:15.

Bhatnagar, P.S. and S.P. Tiwari. 1991. Soybean improvement through mutation breeding in India. In: Plant Mutation Breeding for Crop Improvement, Vol. 1 IAEA, Vienna. pp. 381-384.

Ozbek, N., C. Atak, A.S. Atila and Z. Sagel. 1991. Radiation induced mutations for yield and oil content in soybean. In: Plant Mutation Breeding for Crop Improvement, Vol. 2 IAEA, Vienna. pp. 193-198.

Table 1. Stem tunnelling and grain yield in soybean mutants of "PK 472"

S No.	Mutants	Stem tunnelling (%)@	Rating	Grain Yield (kg/ha)	Flower color	DTM
1.	15Kr+UV '31'	24.60 (29.73)	R	3560	Purple	109
2.	15Kr+UV '69-4'	27.89 (31.86)	MR	3351	Purple	112
3.	20Kr+UV '75-7'	25.18 (30.10)	MR	2755	Purple	110
4.	20Kr+UV '84-16'	30.29 (33.36)	LR	3259	White	109
5.	20Kr+UV '84-14'	32.56 (34.80)	S	3183	White	112
6.	20Kr+UV '82-7'	26.74 (31.13)	MR	3800	White	109
7.	20Kr+UV '85-1'	26.57 (31.01)	MR	3448	White	110
8.	20Kr+UV '85-10'	32.04 (34.46)	LR	3277	Purple	111
9.	20Kr+UV '82-16'	34.76 (36.12)	HS	3858	White	111
10.	20Kr+UV '72-12'	23.13 (28.72)	HR	3979	White	110
11.	20Kr+UV '82-5'	26.97 (31.26)	MR	3092	White	111
12.	20Kr+UV '72-1'	29.69 (32.68)	LR	3178	White	111
13.	20Kr+UV '30'	30.24 (33.36)	LR	3567	White	111
14.	PK 472 (control)	27.83 (31.83)	MR	3281	White	112
	LSD (P=0.05)	(2.35)		169.34		

Table 2. Stem tunnelling and grain yield in soybean mutants of "Bragg"

S No.	Mutants	Stem tunnelling (%)@	Rating	Grain Yield (kg/ha)	Flower color	DTM
1.	15Kr+UV '72-2'	26.33 (30.85)	MR	3401	White	111
2.	20Kr+UV '93-3'	27.71 (31.75)	MR	3052	White	112
3.	20Kr '31-6'	27.81 (31.80)	MR	3319	White	111
4.	20Kr '14-3'	23.31 (28.86)	R	3377	White	113
5.	20Kr '31-17'	27.84 (31.84)	MR	3548	White	112
6.	20Kr+UV '95-10'	27.58 (31.66)	MR	3896	Purple	106
7.	25Kr '39-17'	24.40 (29.60)	MR	3430	White	112
8.	Bragg (control)	39.80 (39.09)	HS	3216	White	113
	LSD (P=0.05)	(2.61)		122.2		

DTM - Days to maturity
@ - Angular transformed values are given in parantheses.

Amar N. Sharma
P.S. Bhatnagar
R.N. Singh

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Indore 452 001, Madhya Pradesh
INDIA

Quantifying girdle beetle resistance in soybean

Introduction: Girdle beetle (Obereopsis brevis Swed., Lamiidae: Coleoptera) is one of the key pests of soybean, causing more than 50 percent yield losses (Kunda and Trimohan, 1986). Due to its intricate life cycle, its management is rather difficult. Specific efforts to develop girdle beetle resistant/tolerant soybean varieties has yet to gain momentum in India. However, some resistant/less susceptible sources have been reported (Anonymous, 1984; Bhattacharjee and Goswami, 1986; Kashyap et al., 1980; Sharma, 1992).

According to the existing screening procedure followed under All India Coordinated Research Project on Soybean (AICRPS) (Anonymous, 1992), a genotype is rated resistant or susceptible to girdle beetle on the basis of percent infested plants (i.e. plants showing girdles made by the females for egg laying) derived from 25 randomly selected plants. This apparent infestation level does not appear to be the precise measure of actual damage and yield loss. This is because all the infested plants do not succumb to the injury, owing to some inherent resistance.

In breeding a variety resistant to girdle beetle, breeders would be required to know the level of resistance in the parents and its inheritance in successive generations. The objective of this study was to identify girdle beetle resistant sources and to quantify resistance in some germplasm lines/cultivars.

Materials and Methods: Two sets of 16 genotypes were planted on June 19, 1993 in two replications. One set was given only water spray (T1), while other set (T2) received phorate 10G @ 1 kg a.i./ha (pre-sowing soil application), quinalphos 25EC (before flowering) and monocrotophos 36SC (at pod setting) both @ 800 ml/ha. Girdle beetle infested polants were tagged for futher observations. Difference in number of infested plants in unprotected and protected plots was used to calculate percent reduction in infestation by using chemical insecticides. After senescence, number of plants cut down by the grubs (typical damage symptom) were counted and expressed in percentage.

Percent resistance was then calculated on the basis of plants cut by the grubs in unprotected plots by using formula:

$$\% \text{ Resistance} = \frac{\text{No. of infested plants - No. of plants cut by grubs}}{\text{No. of infested plants}} \times 100$$

Results and Discussion: Average girdle beetle infested plants per plot varied from 6.5 (T1) and 3.0 (T2) in DS 396 to 26.5 (T1) and 18.0 (T2) in Soja savana as more preferred for egg laying despite insecticide application. Among the four check varieties, Gaurav had maximum infestation followed by NRC 2, Punjab 1 and JS 335. Infestation could be reduced significantly by using chemical insecticides. Maximum reduction of 74.61% was achieved in GC 60068-9, and minimum in Soja savana (32.28%). This differential response of genotypes to insecticides in reducing girdle beetle infestation could be attributed to factors like leaf area, canopy structure, root growth pattern (all influencing uptake of insecticides) and specific genetic makeup.

Under unprotected conditions, TG x 814-54D had minimum percent cut plants, thereby exhibiting maximum resistance of 87.86%, whereas, Gaurav had maximum percent cut plants and thus least resistance (3.84%). This deviation of susceptibility from Soja savana to Gaurav, on two different criteria, indicates that infestation (egg laying) and the damage are not necessarily correlated. Further, it also indicates that female egg laying preference does not always ensure successful development of teh grubs. Among other germplasms, which showed significantly higher percent resistance than the cultivated varieties, important ones are L-129, DS-396, TG x 855-53D, TG x 1973 - 55E and TG x 824-34D.

Percent resistance obtained on the basis of plants not showing typical cutting symptoms in unprotected conditions represent the level of resistance inherently present in a particular genotype. For further confirmation, single plant progenies of all "resistant" plants could be tested under field and/or cage conditions. Further studies to find out the factors influencing egg laying and development of grubs are warranted. Tolerant sources giving good yields under both unprotected and protected conditions, would be ideal substitute for high yielding but susceptible released varieties.

References:

Anonymous. 1984. Proceedings of 15th Annual Workshop of AICRPS. BAU, Ranchi, Bihar. April 5-7, 1984. GBPUA & T, Pantnagar, pp. 55-59.

Anonymous. 1992. Proceedings of 23rd Annual Workshop of AICRPS. UAS, Bangalore, Karnataka. May 7-9, 1992. NRC for Soybean, Indore. pp. 48-53.

Bhattacharjee, N.S. and K. Goswami. 1986. Preliminary studies on the incidence of girdle beetle O. brevis in soybean. Indian J. Ent., 46:126-128.

Kashyap, N.P., S.F. Hameed and D.N. Vaidya. 1980. Preliminary studies on the varietal susceptibility of soybean to Nupserha nitidior pic. var. atripenis Trop. Grain Legu. Bull. 13/14:50-52.

Kundu, G.G. and Trimohan. 1986. Effect of infestation by the girdle beetle. O. brevis on plant parameters contributing yield in soybean crop J. Ent. Res., 10:57-62.

Sharma, A.N. 1992. Multiple insect resistance in soybean Glycine max (L) Merr. under field conditions. FLCG Newsl. 18:5.

Amar N. Sharma

Table 1. Resistance to girdle beetle in Soybean

S. No.	Genotypes	Ave. infested plants #		% Reduction in infestation @	% Plants cut by grubs @		% Resistance (on the basis of T1) @
		Unprotected (T1)	Protected (T2)		Unprotected (T1)	Protected (T2)	
Check varieties							
1.	Gaurav	24.5 (5.00)fg	12.5 (3.60)	49.08 (44.48)abc	96.19 (81.94)	20.83 (27.06)ab	3.84 (8.05)
2.	JS 335	12.5 (3.60)bcde	7.5 (2.82)d	39.74 (39.00)a	59.59 (50.52)fg	14.28 (22.22)ab	40.40 (39.73)bc
3.	NRC 2	14.5 (3.86)de	7.5 (2.82)d	48.07 (43.88)abc	75.96 (60.67)gh	26.66 (30.90)b	24.04 (29.36)ab
4.	Punjab 1	17.5 (4.91)ef	4.0 (2.10)ab	72.46 (58.50)bc	57.14 (49.15)ef	25.00 (22.50)ab	42.86 (40.84)cd
Germplasm lines							
5.	DS 396	6.5 (2.64)a	3.0 (1.87)a	52.38 (46.45)abc	20.83 (27.06)abc	0.00 (0.00)a	79.16 (62.94)fgh
6.	GC 60068-9	14.0 (3.80)de	3.5 (1.99)a	74.61 (59.86)c	80.00 (63.44)h	0.00 (0.00)a	20.00 (26.56)a
7.	L 129	12.5 (3.59)abcd	7.5 (2.82)d	36.66 (36.72)a	19.64 (26.11)abc	7.14 (11.11)ab	80.36 (63.89)fgh
8.	Soja savana	26.5 (5.19)g	18.0 (4.29)	32.28 (34.63)a	84.58 (67.21)h	0.00 (0.00)a	15.36 (22.78)a
9.	TG x 239-30D	15.5 (3.96)e	6.0 (2.54)bcd	59.77 (50.65)abc	50.00 (45.00)ef	12.50 (15.00)ab	50.00 (45.00)cd
10.	TG x 342-536D	13.5 (3.73)cde	7.0 (2.73)d	48.33 (44.05)abc	61.25 (51.50)fg	10.00 (13.28)ab	38.75 (38.49)bc
11.	TG x 539-2F	13.5 (3.72)cde	6.0 (2.51)bcd	56.82 (48.94)abc	40.00 (39.10)de	20.00 (19.61)ab	60.00 (50.89)de
12.	TG x 814-26E	12.0 (2.60)a	6.5 (2.64)cd	47.76 (43.70)abc	31.66 (34.22)cd	0.00 (0.00)a	68.33 (55.77)ef
13.	TG x 814-54D	7.5 (2.82)ab	4.0 (2.14)abc	46.43 (42.96)abc	12.14 (20.33)a	0.00 (0.00)a	87.86 (69.67)h
14.	TG x 824-34D	12.5 (3.59)bcd	6.5 (2.64)cd	45.00 (41.99)ab	26.78 (31.16)bcd	8.33 (12.06)ab	73.21 (58.83)efg
15.	TG x 855-53D	9.0 (3.07)abcd	4.0 (2.10)ab	56.25 (48.62)abc	12.50 (20.70)ab	0.00 (0.00)a	87.50 (69.30)gh
16.	TG x 1073-55E	8.5 (2.93)abc	3.0 (1.87)a	57.50 (49.61)abc	26.66 (30.90)bcd	0.00 (0.00)a	73.33 (59.10)efg

Transformed to $\sqrt{n+0.5}$, @ Transformed to corresponding arcsin values
Transformed values are given in parantheses.
Means within the column, followed by same letter do not differ significantly at P=0.05.

PUNJAB AGRICULTURAL UNIVERSITY
Dept of Seed Sci & Technology
Ludhiana (India) - 141 004

Evaluation of Soybean Genotypes for Seed Storability

Soybean [Glycine max (L) Merrill] seed is a rich source of both protein and oil and meets about 60 per cent of the world's supply of vegetable protein and 30 per cent of the oil (Foreign Agricultural Service, 1985). Its seeds are classified among poor storers with short life span in the warm humid tropics.

Keeping in view the importance of soybean, its twenty diverse genotypes were screened for seed storability to identify poor and good seed storer genotypes.

Material and Methods: The seed of twenty soybean genotypes produced under the identical conditions were stored in cloth bags and germination was tested in quadruplicate of 100 seeds each at monthly intervals (ISTA, 1985). The number of hard seeds were noted at the end of germination.

Results and Discussion: Seed storability is the period for which they retain their germinability up to a minimum prescribed level which is 70% in soybean. Different crop species have specific life spans; similarly not all cultivars or individual seeds within a genetic group are destined to survive for the same duration under specified environmental conditions.

Based on the seed storability the genotypes were divided into the following 3 groups:

GROUPS	
Poor storer with 6 months storability:	PK 472
Intermediate storers with:	SL, 178, SL 96,SL 208,SL 152, SH-84-25, DS 108, SL 133, PB 1, PK 416, SL 170, Bragg, PK 564, SL 104, DS 9, PK 853
Good storers with 16 months storability:	SL 107, SL 144, SL 127

Further, genotypes SL 170, SL 178, SL 191, SL 127 and PK 416 had significant proportion of hardseeds which were dissipated with progressive storage; however, two genotypes *viz.* SL 170 and SL 127 still retained significant hardseeds after 16 months of storage. No definite relationship could be established between hardseeds and storability in soybean as was noticed in a number of leguminous crops (Justice and Bass, 1979).

Only a limited number of studies have been conducted on inheritance of long storage life among cultivars and most of these pertained to maize. Therefore, the present material might be desirable for further study. Further, these observations can be utilized to study the mechanism of seed deterioration where contrasting good and poor storer genotype seeds be compared for their response to aging.

References:

Foreign Agricultural Service. 1985. Foreign Agricultural Circular Fol. 1-85 USDA, Washington, D.C.

ISTA. 1985. International Rules for Seed Testing. Seed Science and Technol. 13: 299-354.

Justice, O.L. and L.N. Bass. 1979. Principles and Practices of Seed Storage. CastleHouse Publications Ltd. pp. 289.

Gurmit Singh
S.S. Gill
T.P.Singh

PUNJAB AGRICULTURAL UNIVERSITY
Dept. of Seed Science and Technology
Ludhiana, India 141 004

Flowering Behavior of Soybean Genotypes

Introduction: Soybean is a short day plant. Garner and Allard (1920) discovered the significance of day length in the flowering behavior of soybeans. Most soybean varieties respond to photoperiod, but they vary widely with respect to critical day length at which flower formation is initiated. Hill, group V cultivar has critical photoperiod of approximately 14.8 hours (Murphy and Porfido, 1977).

Flowering in soybean occurs when day length becomes shorter than the critical photoperiod for the variety. It takes about three weeks between induction and opening of flowers in Biloxi (Borthwick and Parker, 1938 C). Garner (1933), showed that responses to day length are modified by temperature. The primary effect of temperature is on the photoperiod reaction in the leaf blade (Parker and Borthwick, 1943). Roberts (1943) showed that temperature during the dark period is more important than that during light. Whigham et al., 1978, found that minimum temperatures were associated with delays in flowering under tropical conditions. Optimum temperature range for most cultivars i s30-33°C day temperature. Optimum temperature, however, varies with the life of the plant. At certain periods, night temperature has significant influence on development. Good flowering occurred with a leaf temperature of 65°F (18°C) in the dark, but none with temperatures below 55°F. Shanmugasundaram and Tsou (1978), observed that in the tropics and sub-tropics, flowering behavior of cultivars may differ with planting season even though photoperiods at planting are similar.

The present study was conducted at Ludhiana; which is located 30°-56' North latitude and 75°-52'East longitude. Ten genotypes were planted every month beginning from February to August. Date of flowering was recorded for each variety for all the sowing dates. The average hours of sunshine (day length) from first sowing in February to last sowing in August varied from 11 hours four minutes to 13 hours 13 minutes. The average minimum temperature was 9.3°C in February and 25.9°C in August. Similiarly, the maximum day temperature was 19.7°C in February and 32.9°C in August. Figure 1 shows day length and temperature fluctuation for each month from February to October at Ludhiana. From the day length variation, it is clear that the soybean grown in

February, March and April were exposed to increasing day length from sowing to flowering, while those sown in June, July and August were exposed to decreasing day length from sowing to flowering. The minimum temperature for February, March and April sowing was less than 20°C. The maximum day temperature, however, rose from 20.1°C in February to 33°C. For June, July and August sowings, maximum temperature ranged between 32.9°C and 41.1°C. Since the experiment was grown under field conditions, the response of the soybean genotypes measured in terms of days to flowering was a combined effect of day length and temperature. The variation in days to flowering of ten genotypes for seven sowing dates is given in Table 1.

Fluctuations in day length and temperature resulted in variation in days to flowering of a particular genotype over different sowing dates. The highest variation for flowering was observed in the varieties PK 472 and Punjab Soybean Number one. The lowest variation was, however, shown by the varieties, HIMSO 1550 and KB 79. Considering the response of different genotypes to various sowing dates, it was observed that the June sowing caused the least variation in days to flowering among the genotypes studied (Table 1). This may be due to the fact that the critical day length and temperature regime requirement of different genotypes was most appropriately met with July and August sowing also showed less variation for days to flowering among the genotypes. The highest variation among genotypes for number of days to flower was in April sowing followed by March sowing. All the genotypes generally flowered late, when sown in February, which may due primarily to day length being shorter than their critical day length requirement. Low night and day temperature may also be responsible for delayed flowering. the varieties SL 96, HIMSO 1550 and KB 79 showed less variation in their days to flowering over different sowing times while the varieties SL 142, SL 253, Punjab Soybean No. 1 and PK 472 showed higher variation over different sowing times. The varieties BS 1, SH 84-25 and PK 416 showed moderate variability in days to flowering over sowing times. This indicates that varieties showed differential response to the prevailing day length and temperature at different sowing times. The genotypes PK 472 and Punjab Soybean No. 1 flowered later than all other genotypes, particularly during February, March , April and May sowing, possibly due to their higher critical day length requirement than other genotypes.

From this study it can be concluded that the genotypes differed in their response to photoperiod and temperature. Critical day length requirement for PK 472 and Punjab Soybean No. 1 is higher than other varieties. However, prevailing day length and temperature during different sowing periods (February and August) appear to be suitable for the flowering of all the genotypes tested indicating the possibility of growing a successful crop of soybean in summer (February to June) if a photo and thermo insensitive genotype is identified.

References:

Borthwick, H.A. and M.W. Parker.1938 C. Photoperiodic perception in Biloxi Soybeans. Bot. Gaz. 100, 374-387.

Garner, W.W. 1933. Plant Physiol. 8:347-356.

Garner, W.W. and H.A. Allard. 1920. Photoperiod responses of soybeans in relation to temperature and other environmental factors. J.Agr. Res.41. 719-735.

Murphy, G.M. and O.D. Porfido. 1977. Determination del fotoperiod critico em cultivars de soja de distintos groups de madiraccion. 17p. (Trabalho a presentation 5 a Reunion Tecnica Nacional de soja de Miramar, Buenos Aires, Argentina).

Parker, M.W. and H.A. Borthwick. 1943. Influence of temperature on photoperiodic reactions in leaf blades of Biloxi soybean. Bot. Gaz. 104:612-619.

Roberts, R.H. 1943. Science 95:265.

Shanmugasundaram, S. and S.C.S. Tsou. 1978. Photoperiod and critical duration for flower induction in soybeans. Crop Sci. 18:598-601.

Whigham, D.K., H.C. Minor and S.G. Carmer. 1978. Effects of environment and management on soybean performance in the tropics. Agron. J. 70: 587-92.

T.P. Singh

P.S. Madan

P.S. Phul

T.R. Ghai

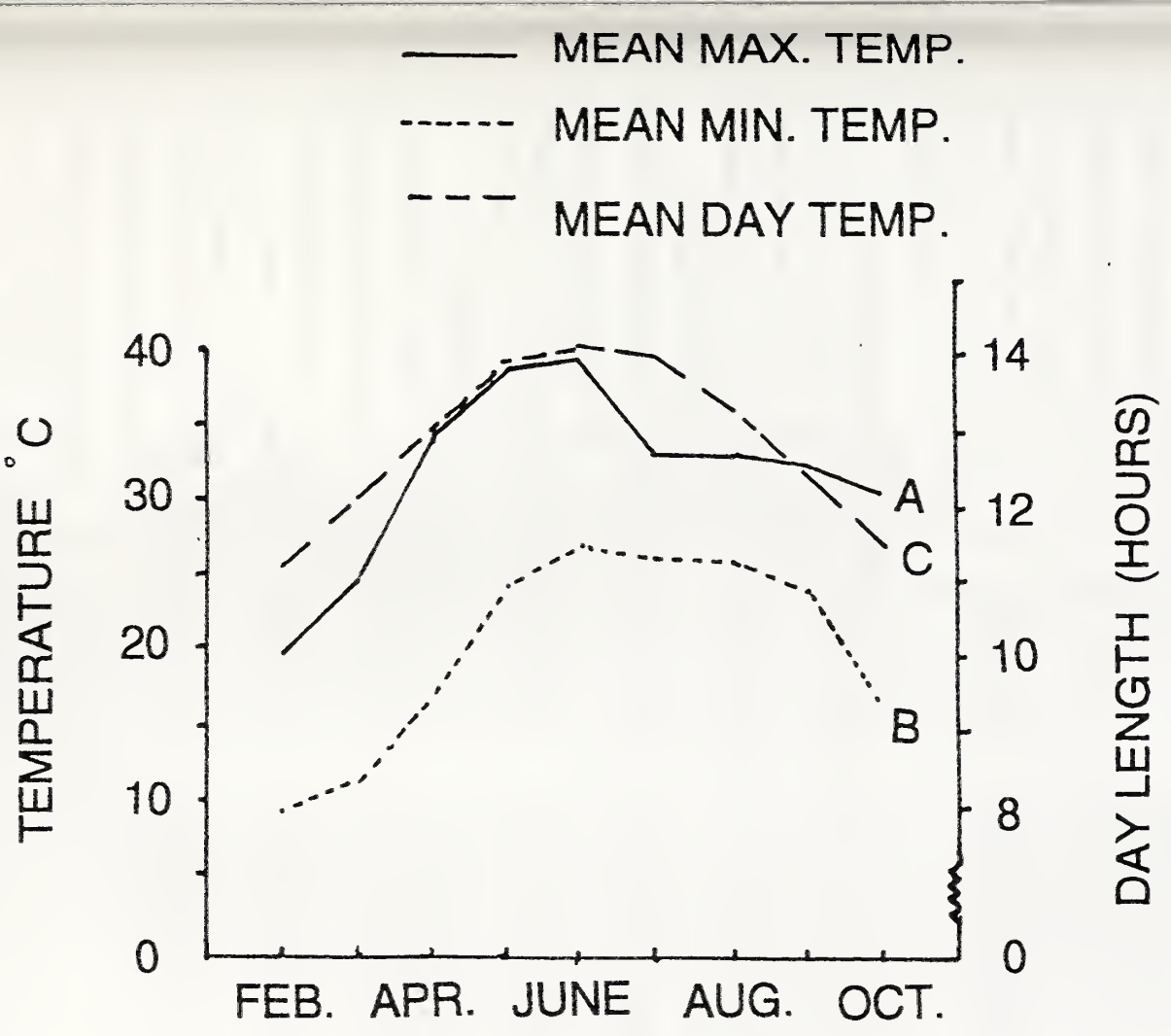


FIG. 1 MEAN, MAXI, AND MINI. TEMP. AND DAY LENGTH FROM FEB. TO AUGUST AT LUDHIANA.

Table 1. Days to flowering of ten genotypes for seven monthly sowings.

Sr. No.	Variety	Days to flowering when sown in:							Mean	Range	σ
		Feb.	Mar.	April	May	June	July	Aug.			
1.	SL-253	64	55	73	69	46	57	44	58.3	44-73	10.2
2.	BS-1	64	49	59	68	46	60	43	55.6	43-68	8.9
3.	PK-472	80	72	72	72	49	58	44	63.9	44-80	12.6
4.	SL-96	60	48	50	66	48	60	44	53.7	44-66	7.6
5.	HIMSO-1550	64	48	49	59	46	55	48	52.7	46-64	6.3
6.	KB-79	66	51	50	61	47	59	49	54.7	47-66	6.7
7.	Punjab Soy-bean No. 1	80	72	77	76	49	60	47	65.9	47-80	12.8
8.	SL-142	62	49	75	70	48	58	43	57.9	43-75	11.0
9.	SH-84-25	63	53	71	68	47	56	48	58.0	47-71	8.8
10.	PK-416	69	52	59	69	48	56	43	56.6	43-69	9.2
	Mean	67.2	54.9	63.5	67.8	47.4	57.9	45.3			
	Range	62-80	48-72	49-77	59-76	46-49	55-60	43-49			
	σ	6.8	8.8	10.7	4.7	1.1	1.8	2.3			

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Indore - 452001, India

Transgressive segregants with four-seeded pods in soybean

Introduction: Enhancement of overall seed number per unit area could bring about increased productivity in soybean. Yield components viz. pods per plant and seeds per pod were reported to be more important than others (Herbert and Litchfield, 1985; Prabhakar and Tiwari, 1993). Information available on increasing the number of seeds per pod is scanty. Usually there are two to three seeds in a single soybean pod. An interspecific derivative reported to have about 30% 4-seeded pods had small seeds. The present report brings about the success achieved in breeding lines with 4-seeded pods and acceptable seed size by using the variability among the cultigen [G. max (L.) Merr.].

Materials and Methods: The varieties and strains viz. 'Kalitur', 'PK 472', 'PK 308', 'NRC 1', 'NRC 2' and 'NRC 3' and subsequent generations derived after hybridization was studied at Indore from the years 1988 to 1993. Mean values for frequency of 1- to 4- seeded pods were computed.

Results and Discussion: The indigenous black-seeded 'Kalitur' exhibited residual heterogeneity and some plants possessing up to 20% 4-seeded pods were selected which bred true. The frequency of one-seeded pods in these selections was negligible. The mean percentage occurrence of 2- , 3-, and 4- seeded pods was 18.2, 66.0 and 15.8%, respectively.

The cross 'Kalitur' x 'PK 472' was effected. Plants with 4-seeded pods were not observed in F_1 and F_2, but in F_3. These were semi-indeterminate and black-seeded. The highest frequency of four-seeded pods was 28%. Mean percentages of 2-, 3-, and 4-seeded pods were 16.7, 65.4 and 17.9%, respectively.

In order to achieve yellow-seeded lines with 4-seeded pods, the parent 'Kalitur' was replaced by its yellow-seeded mutant 'NRC 1', developed at N.R.C.S., and the cross was repeated. This cross i.e. 'NRC1' x 'PK 472' did not throw any 4-seeded segregants. The reason could be that the mutant, unlike its parent 'Kalitur', has less frequency of 3-seeded pods. The mean frequencies of 1-, 2- and 3-seeded pods in the mutant were 3.0, 84.2 and 12.8%, respectively.

Success in breeding yellow-seeded lines with up to 30% 4-seeded pods was, however, achieved in F_3 and F_4 generations of the cross-combinations 'PK 308' x 'NRC 2' (an induced mutant of 'Bragg') and 'PK 308' x 'NRC 3' (a selection from local collection). Besides 4-seeded pods, the rest of the pods in these lines were mostly 3-

seeded. None of the parents involved in the crosses possess 4-seeded segregants themselves, although 'PK 308' and 'NRC 3' bear a high frequency of 3-seeded pods. The later-generation 4-seeded segregant had excelled their parents and thus represent transgressive segregation for the character. The selections bred true for the character. Stable lines with acceptable commercial features have been developed. The lines have 100-seed weight ranging from 9 to 12 g in contrast to the low seed weight of about 8 g in the earlier lines developed through interspecific hybridization with Glycine soja Sieb & Zucc. (Gour et al., 1992), although the frequency of 4-seeded pods in these lines was comparable with the latter.

Conclusion: Yellow-seeded soybean lines were developed with up to about 30% 4-seeded pods in addition to the black 4-seeded lines earlier derived from the indigenous 'Kalitur' by us. The lines have commercially acceptable features unlike the interspecific derivatives which suffer from small-seededness. The lines appeared as transgressive segregants after hybridization of cultigen parents, which do not bear 4-seeded pods. Varieties 'PK 308', 'NRC 3' and 'Kalitur' were identified as desirable parents for obtaining segregants with 4-seeded pods. Further studies are continuing.

References:

Gour, V.K., A.K. Mehta and S.K. Mehta. 1992. A line with four-seeded pods derived from an interspecific hybrid in soybean. Plant Breed. 108:260-262.

Herbert, S.T. and C.V. Litchfield. 1982. Partitioning soybean seed yield components. Crop Sci. 22:1074-1079.

Prabhakar and S.P. Tiwari. 1993. Multiple regression analysis for seed yield in soybean. Soybean Genet. Newsl. 20:70-72.

S.P. Tiwari
P.S. Bhatnagar

NATIONAL BUREAU OF PLANT GENETIC RESOURCES
Regional Station, Akola, Maharashtra
444 104, India

Screening of soybean germplasm for photoperiodic insensitivity under natural conditions in Vidarbha region of Maharashtra, India

Introduction: Photoperiodic insensitivity has been reported in soybean by Yoshida (1952), Pohjakallio and Antila (1957), Criswell and Hume (1972), Shanmugasundram (1974,1978) . Early varieties of soybean have been found to be less sensitive to photoperiodic response than late varieties (Johanson et al., 1960). Delay in days to flowering for 4-40 days was reported for screening of photoperiodic insensitivity under natural conditions in soybean (Ram et al., 1982). In India, soybean is mainly grown in the rainy season. The Vidarbha region of Maharashtra has about 0.225 million hectare land under soybean cultivation in the rainy season only and it is substantially increasing in those areas where cotton yields were poor. Farmers of the Vidarbha region were obtaining the poor yields of soybean in the winter/spring season, because of growing unsuitable soybean cultivars. Hence, 3000 soybean accessions were tested in the winter/spring season with the objective to screen for photoperiodic insensitivity under natural conditions in the Vidarbha region of Maharashtra, India.

The Vidarbha region is located at 20-21.5° N lat. and 76-80° E in the central part of India. This area is near to the soybean growing belt of the country. The soil is black cotton soil derived mostly from basalt rocks. Because of suitable climate and soil conditions, it is possible to grow soybean as a second crop after sorghum, groundnut and cotton harvests.

Materials and Methods: The study was conducted at NBPGR, Regional Station, Experimental Farm, Akola, Maharashtra in the winter/spring season of 1992-93. Three thousand accessions of soybean comprised of exotic and indigenous genotypes were grown on the 7th of December, l992 in augment block design, with 3 check varieties i.e. 'MACS13,' 'Bragg' and 'Clark 63' (high yielding cutivars). The row length was one meter and row to row distance was 50 cm. Just after planting the seed material, flood irrigation was applied for uniform germination. A total of 4 irrigations was applied till maturity, with an interval of 15-20 days. Data were recorded on days to flower, plant height, days to maturity, pods/plant, yield/row, 100-seeds weight and calculated

yield/hectare. The climatic data over the growing period in Akola in 1992-93 are given in Table 1.

Table 1. The climatic data over growing period in Akola in 1992-93.

Months	Rainfall (mm)	No. of rainy days	Temp max.	Temp. min.	Rel Humid. 8.30 hours	Rel. Humid 14.30 hours	Sun-shine hours
Dec, 1992	----	----	29.8	8.0	73	23	9.4
Jan, 1993	----	----	31.3	8.9	73	21	9.5
Feb, 1993	17.2	2	32.9	9.7	58	15	9.8
March, 1993	65.4	3	34.9	15.8	61	25	9.9
April, 1993	----	----	41.2	24.2	29	12	10.1
May, 1993	----	----	43.1	27.3	38	16	10.0

Results and Discussion: Most rainy season varieties of soybean do not do well in the winter/spring season. However, if they are sown in the winter/spring season, both the flowering and maturity period are prolonged. The genotypes which fit well under short-day conditions of 9-10 hours were considered for photoperiodic insensitivity. The photoperiodic insensitivity of each accession was measured by degree of delay in flowering and maturity between rainy and winter/spring season. Flowering was obtained 1-55 days delay and maturity 5-45 days delay as compared to rainy season's flowering and maturity in 3000 accessions of soybean. Forty-six high yielded accessions were selected which matured 5-15 days delay in winter/spring season as compared to their normal growing rainy season (Table 2). Similar results were reported for photoperiodic response in soybean based on degree of delay for flowering and maturity either between natural and artificially created extended photoperiod or between rainy and spring season (Nissly et al., 1975, Shanmugasundram, 1978 and Ram et al., 1982).

The yield ranged from 80 (EC251480) - 140 g/row (EC291391) among 46 accessions selected from 3000 accessions of soybean considering low photoperiodic sensitivity and high yield. Highest yield was obtained from EC291391 (140 g/row) followed by EC14483, EC38137, EC117633, IC118237, IC118288, IC118369, IC118440, IC118449, and IC118507 (Table

2). Among 46 low photoperiodic sensitive accessions, days to flower ranged from 37-68 days and days to maturity 92-125 days. The early maturing accession was EC280131 (90 days) with determinate growth habit. Plant height varied between 20.5-120.0 cm. The accessions with low plant height (20-30 cm) were poor in yield as compared to the accessions with more than 40 cm height. Pods/plant ranged from 15.5-58.5. Highest number of pods/plant was obtained from EC38137, with 58.5 pods/plant, whereas the lowest from EC118601, with 15.5 pods/plant. Highest 100-seeds weight was obtained from EC289099, with 14.8g and lowest from EC280131, with 6.4g. The majority of high yielded accessions have more than 8.0g hundred seeds weight and semideterminate growth habit.

Conclusion: The accessions identified for low photoperiodic insensitivity based on 5-10 days delay in maturity as compared to rainy season maturity were EC251483, EC280129, EC280130, EC280131, AG12/14-E, TGX85-B-81-180, IC118238, IC118240, IC118304, IC118308, IC118454, IC118468, and IC118601. However, high yielded accessions viz. EC14483, EC38137, EC117633, EC291391, IC118237, IC118288, IC118369, IC118440, IC118449 and IC 118507 which showed 11-15 days delay in maturity as compared to their normal rainy season maturity. These accessions either can be used directly for double crop soybean or generating breeding material for photoperiod insensitivity.

References:

Criswell, J.G. and D.J Hume. 1972. Variation in sensitivity to photoperiod among early maturing soybean strains. Crop Sci. 12:657-660.

Johnson, H.W., H.A. Brothwick and R.T Seffel. 1960. Effect of photoperiod and time of planting on rates of development of the soybean in various stages of life cycle. Bot Gaz. 122:77-95.

Pohjakallio O. and S. Antila. 1957. Photoperiodic characters of some bred strains of soybeans. Maataloustiet Aikak. 29:113-120. (Field Crop Abstr. 11:186. 1958).

Ram, H.H., K. Singh, Pushpendra and V.D. Verma. 1982. Screening for photoperiod insensitivity under natural conditions in soybean. Soybean Genet. Newsl. 9:43-44.

Shanmugasundram, S., T.S. Toung, T.D. Liou and H.B.E. Wu. 1974. Photo period insensitivity in soybean. Agron. Abstr. (Chicago, IL). p. 78.

Shanmugasundram, S. 1978. Variation in the photoperiodic response to flowering in soybean. Soybean Genet. Newsl. 5:91-94.
Yoshida, S. 1952. Photoperiodic responses in soybean plants under long day conditions with supplemental illumination of different intensities at night (preliminary report). Proc Crop Sci. Soc. Jpn. 21:127-128.

V.D. Verma
B.V. Sawaji

Table 2. Promising accessions identified for photoperiodic insensitivity in 3000 accessions of soybean at Akola, during winter/spring 1992-93.

Acc. no.	Day to flr	Days to mat	Plant height (cm)	Pods/ plant	100-seeds wt. (g)	Gro-wth habit	Yield/ row	Calcu-lated yield (Q/ha)
EC14483	68	125	81.3	32.4	10.0	ID	130	26.0
EC34146-A	55	110	55.5	30.1	11.1	SD	105	21.0
EC37110	60	110	35.5	20.5	10.0	SD	100	20.0
EC37190	60	110	38.7	21.5	9.5	SD	100	20.0
EC38137	58	100	63.4	58.5	8.3	SD	130	26.0
EC39031	57	107	43.3	57.3	10.4	SD	100	20.0
EC39047	55	122	83.1	53.5	10.1	SD	110	22.0
EC39494	67	116	107.6	36.3	8.5	ID	110	22.0
EC41685	60	120	52.1	36.0	8.8	SD	115	23.0
EC76736	55	105	30.3	45.0	13.4	D	100	20.0
EC93404	43	105	35.3	28.0	9.2	SD	110	22.0
EC117633	63	121	72.3	34.0	10.7	SD	130	26.0
EC251323	50	107	55.3	36.1	11.5	SD	115	23.0
EC251480	42	103	27.7	23.3	12.4	D	80	16.0
EC251482	37	100	30.0	24.0	13.2	D	95	19.0
EC251483	50	105	43.3	52.0	12.0	D	100	20.0
EC280129	50	105	25.0	30.1	11.8	D	100	20.0
EC280130	47	92	28.7	18.0	9.6	D	90	18.0
EC280131	45	90	20.5	19.3	6.4	D	90	18.0
EC289099	50	105	61.3	32.4	14.8	SD	125	25.0
EC291391	65	122	80.4	32.1	10.0	SD	140	28.0
AGS12/14-E	60	108	89.7	38.3	9.5	SD	120	24.0
TGX85B-81-180	50	100	63.4	35.7	7.3	SD	110	22.0
IC18759	56	116	37.0	24.1	6.9	SD	100	20.0
IC96316	65	115	22.3	32.6	9.6	D	105	21.0
IC118237	60	104	68.0	44.8	11.4	SD	130	26.0
IC118238	57	104	56.3	40.2	11.8	SD	125	25.0
IC118240	57	108	55.3	36.3	11.8	SD	125	25.0
IC118288	56	116	50.5	38.1	9.8	D	130	26.0
IC118304	58	108	66.6	21.4	8.6	SD	125	25.0
IC118308	56	106	62.3	24.7	12.0	D	110	22.0
IC118355	67	125	64.0	24.8	7.3	SD	110	22.0
IC118369	55	105	40.1	51.0	9.5	D	130	26.0
IC118428	55	110	59.3	24.8	8.5	SD	100	20.0
IC118440	60	125	55.0	31.6	8.0	SD	130	26.0
IC118443	58	112	77.6	30.4	8.3	SD	125	25.0
IC118449	56	115	47.0	58.4	8.8	SD	130	26.0
IC118452	61	117	53.3	31.6	8.2	SD	110	22.0
IC118454	54	110	35.5	23.6	12.5	D	120	24.0
IC118460	55	104	37.4	24.6	8.3	ID	105	21.0
IC118463	63	118	83.4	42.0	9.5	D	125	25.0
IC118468	55	103	43.3	25.6	8.5	SD	100	20.0
IC118507	63	125	40.0	27.6	8.3	SD	130	26.0
IC118512	59	115	120.0	27.4	8.3	ID	115	23.0
IC118477	53	108	38.0	19.6	8.0	D	100	20.0
IC118601	59	108	43.2	15.5	11.9	D	100	20.0

D= Determinate
SD=Semi-Determinate
ID=Indeterminate

AGRICULTURAL UNIVERSITY
Department of Genetics and Plant Breeding
60-625 Poznan ul. Wojska Polskiego 71 C.
Poland

1) Variation of morphologic characteristics of soybean genotypes

Environmental factors have an impact on expression of many essential characteristics of soybean plants (Jaranowski et al. , 1983, Gretzmacher, 1977). For this reason, specific reaction of various soybean genotypes to Polish conditions (long daylength, variable temperature during growing period, early autumn coolness, short growth period etc.) should be investigated. Estimation of variation of soybean characteristics may contribute to more efficient selection and hasten releasing of new and improved cultivars.

In this study the attempts to determine variation of morphological characteristics of soybean genotypes, originated from different geographic areas, were carried out in Polish conditions.

Plant material and methods: In the study 25 genotypes of soybean were investigated. Their descriptions (name, maturity group, and origin) are given in Table 1. The experiments were carried out in 1976, 1977 and 1979 on the field of Agricultural Experiment Station at Swadzim near Poznan. The experiments were arranged in partially balanced quadratic lattice design with three replications. Two seeds per hill were sown manually in the space of 10 X 30 cm. After emerging seedlings were thinned to one plant per hill. Four row plots (1.2 X 1.5 m, interrow distance 0.3 m) were applied. Morphologic characteristics: plant height, branch number per plant, and level of the lowest pod setting were taken from 20 ripened plants randomly selected from every plot. The gathered data were used for statistic calculation by means of multivariate analysis of variance.

Results and discussion: Plant development depended on genotype, thermic and water conditions in consecutive growing seasons (Konieczny et. al., 1992). The plant morphotype was generally determined by plant height and branching. Plant height depended much on genotype. Fiskeby V plants were the shortest and the tallest ones in PI 332899 and Vansoy. The smallest variability of plant height was observed in Altona, and the greatest in PI 238923 (Table 2). The growth type was an important factor in the variability of plant height. Determinate and indeterminate growth type influenced other characteristics, too. (Martin and Wilcox, 1963).

Plant ability to produce branches depended on particular genotypes (Table 2). Amurskaya and Norchief plants produced the lowest number of branches per plant and Warszawska and PI 179822 produced the highest number of branches per plant. The smallest variability in branch number per plant was found in PI 232997 and the greatest in PI 231172.

The level of lowest pod set (harvesting space) is important characteristic. It influences usefulness of given cultivar for mechanical harvesting. The genotypes tested were highly differentiated by this characteristic (Table 2). The lowest pod setting was observed in PI 196526 and the highest in PI 232997. The smallest variability of this characteristic was found in Mazowiecka and PI 179822 and the greatest in PI 196525.

Plant height, branch number per plant and the lowest pod set level depend on genotype and may be modified by applied planting density and pattern. In the north latitude early soybean cultivars usually produce relatively short plants which tend to set pods near the soil surface (Muszynski and Jaranowski, 1983). To establish sufficient harvest space the investigations on various density of planting should be carried out in Polish conditions. Highly differentiated morphological characteristics of tested genotypes showed that crossing them and selecting in their offspring may be a proper way for selecting recombinants with optimal morphologic appearance, resistant to lodging, bearing sufficient seed yield and easy for mechanical harvesting.

References:

Gretzmacher, R.F. 1977. Spacing and soybean breeding. Soybean Genet. Newsl. 4: 33-36.

Jaranowski, J., H. Skorupska, G. Konieczny, A. Muszynski and L. Torz 1983. Charakterystyka zmiennosci wazniejszych pod wzgledem gospodarczym cech uzytkowych soi (Characteristics of variability of some economically important useful features of soybean). Zesz. Probl. Post. Nauk Roln. 253:7-24.

Konieczny, G., A. Muszynski and J. Nawracala. 1992. Variation and heritability of phenological characteristics of soybean genotypes in Polish conditions. Soybean Genet. Newsl. 19: 78-85.

Martin, R.J. and R.J. Wilcox. 1973. Heritability of lowest pod height in soybeans. Crop Sci. 13:201-203

Muszynski, A. and J. Jaranowski. 1983. Interrelationships between traits as a basis for estimation of selection value in soybean [Glycine max (L.) Merrill]. Genetica Polonica 24-1:51-58.

Grzegorz Konieczny
Adam Muszynski
Jerzy Nawracala

Table 1. Soybean genotypes selected out for experimentation.

Number	Genotype	Maturity group	Origin
1	PI 250 002	00	Belgium
2	Altona	00	Canada, Ontario
3	PI 153 299	00	Belgium
4	Mazowiecka II	0	Poland
5	PI 291 320B	0	China
6	Tiara	I	unknown
7	PI 257 431	00	Germany
8	Herb-622	I	unknown
9	PI 154 193	00	Holland
10	Vansoy	0	Canada, Ontario
11	PI 180 524	00	Germany
12	PI 238 923	00	Czecho-Slovakia
13	PI 161 431B	00	Sweden
14	Warszawska	0	Poland
15	PI 332 899	0	Hungary
16	Norchief	0	USA
17	PI 231 172	00	Sweden
18	PI 180 508	00	Germany
19	Amurskaya	I	Russia
20	Fiskeby V	00	Sweden
21	PI 196 525	00	Sweden
22	PI 232 997	00	Germany
23	PI 189 900	0	France
24	PI 196 526	00	Sweden
25	PI 179 822	0	Germany

Table 2. Means (x) and coefficients of variation (V) of plant height, branch number per plant and level of lowest pod set of genotypes over 1976, 1977 and 1979.

No.	Genotype	Plant height		Branch no.		Lowest pod	
		X	V [%]	X	V [%]	X	V [%]
1	PI 250 002	58.9	6.7	3.8	10.3	8.3	4.9
2	Altona	59.2	3.6	2.4	3.7	7.3	1.8
3	PI 153 299	67.9	6.6	3.9	3.5	7.5	1.8
4	Mazowiecka II	58.2	4.0	3.9	4.1	7.1	0.1
5	PI 291 320B	75.0	9.1	3.4	6.6	7.0	13.0
6	Tiara	58.7	5.2	3.7	5.8	6.8	7.4
7	PI 257 431	63.1	7.5	3.7	3.2	12.3	5.2
8	Herb-622	41.6	9.1	3.8	8.7	6.2	4.7
9	PI 154 193	57.0	5.0	4.0	5.9	13.7	0.0
10	Vansoy	81.7	8.5	3.1	2.8	11.6	2.5
11	PI 180 524	54.2	8.5	2.5	7.4	7.6	12.3
12	PI 238 923	45.7	13.3	3.7	6.9	6.8	10.4
13	PI 161 431B	55.4	5.1	3.0	5.5	7.7	1.7
14	Warszawska	41.2	11.6	4.3	7.6	6.2	12.3
15	PI 332 899	89.3	8.1	2.9	12.5	13.2	7.6
16	Norchief	73.7	6.5	2.3	7.0	9.2	5.0
17	PI 231 172	49.6	7.8	2.4	14.1	7.3	11.6
18	PI 180 508	57.8	6.7	4.2	4.8	9.2	112.5
19	Amurskaya	74.1	7.5	2.3	5.0	11.0	3.3
20	Fiskeby V	35.1	7.8	3.1	7.5	7.6	4.8
21	PI 196 525	61.5	8.8	3.2	5.8	9.8	14.3
22	PI 232 997	64.2	3.8	3.3	1.5	14.0	6.2
23	PI 189 900	49.9	9.2	4.2	12.4	7.0	1.4
24	PI 196 526	41.6	10.8	3.9	8.6	5.2	23.3
25	PI 179 822	57.8	6.3	4.3	5.7	8.8	0.4

AGRICULTURAL UNIVERSITY
Department of Genetics and Plant Breeding
60-625 Poznan ul. Wojska Polskiego 71 C.
Poland

2) Variation of seed yield components of soybean genotypes

When foreign soybean genotypes are planted in northern conditions their seed yield components change greatly (Jaranowski et. al. , 1983; Gretzmacher, 1977). The changes of pod number per plant are the result of specific reaction of foreign soybean genotypes to Polish conditions such as long day length, low temperature during growing period, short growing period, early autumn coolness etc. Estimation of variation of soybean seed yield components in Polish environment may contribute to more efficient breeding and releasing of better yielding cultivars. Introduction of efficient soybean cultivars to northern environments need more research.

In this study attempts to determine variation of seed yield components of soybean genotypes in Polish conditions were carried out.

Plant material and Methods: In the test, 25 differentiated genotypes of soybean were applied. Their descriptions (name, maturity group and origin) are given in Table 1 of the previous report (no. 1) in this issue. The experiments were carried out according to the method described in the same previous report (no. 1). Pod and seed number per plant, one hundred seed weight and seed yield per plant were taken from 20 ripened plants randomly selected from each plot.

Results and Discussion: Seed yield depended on genotype, especially on its plant morphotype, and particular seed yield components. The distribution of pods on plant differentiated genotypes. On average, plants produced pods on about 60% of their nodes. Most of tested genotypes showed a tendency for producing pods on the lower part of the plant stature. Pod number per plant was determined by genotype (Table 1). The greatest pod number per plant was found in Mazowiecka II cultivar and the lowest in PI 291320B. The lowest variability was observed in PI 180508, and the highest in Vansoy. The pattern of variability of seed number per plant was similar to variability of pod number per plant (Table 1).

The differences among genotypes in one hundred seed weight and in coefficients of variation were rather small (Table 1).

The tested genotypes showed wide range in seed yield per plant (11.1g/plant- II - Mazowiecka II, 4.7 g/plant- PI 291320B). Coefficients of variation

range was from 2.2 (Warszawska) to 17.8 (Vansoy). It was interesting that in spite of different particular seed yield components the seed yields per plant for genotypes in consecutive years of experiments were rather similar. It means that seed yield components have some compensation ability and probably react individually to weather conditions which change every year. Description of variability of seed yield components and their interrelationships (Muszhnski and Jaranowski, 1983) may be essential for induction of variability useful for selecting soybean in Polish environment.

References:

Gretzmacher, R.F. 1977. Spacing and soybean breeding. Soybean Genet. Newsl. 4:33-36.

Jaranowski, J., H. Skorupska, G. Konieczny, A. Muszynski and L.Torz. 1983. Charakterystyka zmiennosci wazniejszych pod wzgledem gospodarczym cech uzytkowych soi (Characteristics of variability of some economically important useful features of soybean). Zesz. Probl. Post. Nauk Roln. 253:7-24.

Muszynski, A., J. Jaranowski. 1983. Interrelationships between traits as a basis for estimation of selection value in soybean (Glycine mas (L.) Merrill). Genetica Polonica 24-1: 51-58.

Grzegorz Knoieczny
Adam Muszynski
Jerzy Nawracala

Table 1. Means (x) and coefficients of variation (V) of pod and seed number per plant, hundred seed weight and seed yield per plant of particular genotypes over 1976, 1977 and 1979.

No.	Genotype	Pod no. per plant		Seed no. per plant		100-seed weight		Seed yield per plant	
		x	V [%]	x	V [%]	x	V [%]	x	V [%]
1	PI 250 002	34.5	3.8	50.5	3.0	16.8	0.7	8.2	4.8
2	Altona	29.8	4.4	52.2	5.6	18.7	3.0	9.6	3.8
3	PI 153 299	33.6	10.5	61.0	13.2	17.2	4.2	10.1	9.9
4	Mazowiecka II	40.5	6.9	74.0	8.5	15.3	1.8	11.1	7.0
5	PI 291 320B	19.4	6.5	27.1	10.1	17.5	0.1	4.7	9.7
6	Tiara	35.6	3.5	61.7	6.1	16.1	0.6	9.9	6.8
7	PI 257 431	27.0	5.9	42.9	7.3	18.2	2.4	7.9	8.8
8	Herb-622	30.2	6.2	45.8	7.1	16.1	1.1	7.6	6.4
9	PI 154 193	29.6	4.6	43.8	6.7	13.5	3.9	5.9	3.5
10	Vansoy	23.3	16.1	42.1	19.0	16.5	2.8	6.7	17.8
11	PI 180 524	23.1	6.4	38.7	7.9	21.5	1.2	8.2	6.8
12	PI 238 923	28.5	9.0	43.5	8.6	17.3	3.9	7.6	3.4
13	PI 161 431B	31.9	4.0	47.7	2.7	14.7	4.4	7.2	3.3
14	Warszawska	34.8	5.6	50.0	3.0	16.2	0.9	8.3	2.2
15	PI 332 899	23.3	6.4	41.7	10.1	13.5	9.7	5.5	6.1
16	Norchief	25.9	10.7	43.7	13.6	15.7	4.7	6.5	11.9
17	PI 231 172	24.4	9.5	40.2	9.2	19.6	2.7	8.1	9.9
18	PI 180 508	30.2	1.6	50.7	0.3	18.2	2.4	9.3	2.7
19	Amurskaya	25.2	3.4	45.5	7.6	13.8	4.7	6.2	4.8
20	Fiskeby V	26.0	11.2	43.3	12.8	17.7	4.7	7.6	10.4
21	PI 196 525	29.6	3.4	47.9	3.8	16.9	1.8	7.9	3.6
22	PI 232 997	28.2	5.1	51.3	4.4	18.5	3.7	9.7	3.1
23	PI 189 900	25.5	13.2	39.1	16.9	16.2	6.5	6.6	17.3
24	PI 196 526	23.3	6.0	36.5	6.6	20.3	1.7	7.4	5.4
25	PI 179 822	34.8	11.4	48.6	10.6	14.3	5.7	7.3	12.6

AGRICULTURAL UNIVERSITY
Department of Genetics and Plant Breeding
60-625 Poznan ul. Wojska Polskiego 71 C,
Poland

3) Heritability, discrimination power, year to year variation of some characteristics and main component configuration of soybean genotypes.

Photosensitivity, thermic and water requirements of soybean genotypes determine their usefulness for cultivation in given environmental conditions. Separating genetic from environmental influences on soybean productivity needs more detailed research.

In this study heritibility, discrimination power of some characteristics and main component configuration of genotypes were calculated in the aim to find the most useful implement for soybean selection.

Plant material and methods: In the test, 25 different genotypes of soybean were applied. Their descriptions (name, maturity group and origin) are given in Table 1 of the report no. 1 of this Soybean Genet. Newsl. issue. Biometric measures were carried on 20 ripened plants randomly selected from every plot. Characteristics measured are given in Table 1. The gathered data were used for statistic calculations by means of multivariate analysis of variance.

Results and discussion: Multivariate analysis of variance allowed to reject the general hypothesis about homogeneity of 25 tested genotypes. Analysis of discrimination power of 18 characteristics proved their different contribution to genotypic variability (Table 1). Genotypic distinction of genotypes with regard to the whole set of studied characteristics was determined by the distances dividing the points on the configuration of main components chart (Figure 1).

Ecological requirements of soybean, especially during flowering and pod filling, depend much on genotype (Mackiewicz, 1959); Brown and Chapman, 1960; Holmberg, 1973; Konowa et al., 1975). In the screening of genetic variation of soybean the earliness of ripening was a noticeably criterion in distribution of main components configuration chart. In the classification of breeding materials of soybean, Broich and Palmer (1980) applied dendrographic method, but Shwe et al . (1972) applied analysis of main components. They separated, as a main criterion of genotype distinction, photosensitivity which decides about earliness of ripening.

Considering the most suitable morphotype of soybean plant to Polish conditions it seems that following genotypes may be promising for future crossing:

Fiskeby V, PI 238 923, PI 232 997 which were early enough, developed proper plant morphotype (high pod setting), yielded well and showed proper proportions of seed yield component. Jaranowski et al. (1983) listed coefficients of variation concerning similar genotypes and characteristics. Their data were very close to the results presented in the current report.

Phenotypic variation of growth and flowering periods, plant height, a hundred seed weight and seed number per pod were highly determined by genotype (Table 2). This means that these characteristics should be considered in selection. This is in accord with the report of Haque (1977). The variability, heritability and interrelations of characteristics (Muszynski and Jaranowski, 1983) may be helpful in carrying out effective selection in Polish environment.

Table 1. Discrimination power of examined characteristics.

No.	Characteristic	Discrimination power value
1	Period from planting to flowering	2.8
2	Flowering period*	3.9
3	Pod filling period#	2.4
4	Growth period$	1.2
5	Plant height	1.8
6	Branch number	3.5
7	Level of lowest branch setting	3.0
8	Level of lowest pod setting	3.0
9	Main stem node number	0.9
10	Node number per plant	1.9
11	Fruiting node number	1.7
12	Pod number on upper plant half	2.4
13	Branch pod number	3.8
14	Plant pod number	2.6
15	Plant seed number	2.8
16	Seed number per pod	1.2
17	100 seed weight (g)	1.0
18	Seed yield per plant	1.9

* from beginning to end of flowering

\# from end of flowering to harvest maturity

$ from planting to harvest maturity

Table 2. Means, ranges, variability coefficients and heritability of all characteristics taken over 25 soybean genotypes and years (1976, 1977, and 1979).

No.	Characteristics	Mean	Range	Variability coefficient (%)	Heritability
1	Period from emergence to flowering	45.2	24-69	1.0	0.80
2	Flowering period	24.0	9-57	42.1	0.87
3	Pod filling period	54.4	36-82	14.8	0.58
4	Growth period *	139.9	109.0-171.0	9.5	0.92
5	Plant height (cm)	58.9	24.4-117.8	29.1	0.89
6	Branch number	3.4	1.3-5.8	26.3	0.76
7	Level of lowest branch setting (cm)	4.3	1.6-9.2	34.5	0.90
8	Level of lowest pod setting (cm)	9.5	4.7-19.5	34.1	0.68
9	Main stem node no.	14.3	10.6-20.3	11.7	0.69
10	Node number/ plant	27.4	14.7-43.0	21.3	0.77
11	Fruiting node no.	17.3	6.5-32.7	22.9	0.59
12	Pod no. on upper plant half	13.4	1.6-29.3	36.0	0.17
13	Branch pod number	13.4	2.0-33.5	37.1	0.69
14	Plant pod number	28.7	8.4-55.9	25.6	0.43
15	Plant seed number	46.8	12.7-106.9	29.6	0.34
16	Seed no. per pod	1.6	1.2-2.2	11.2	0.68
17	100 seed weight (g)	16.8	8.7-23.2	14.9	0.83
18	Seed yield/ plant (g)	7.8	2.0-15.5	27. 7	0.47

* Days from sowing to harvest maturity

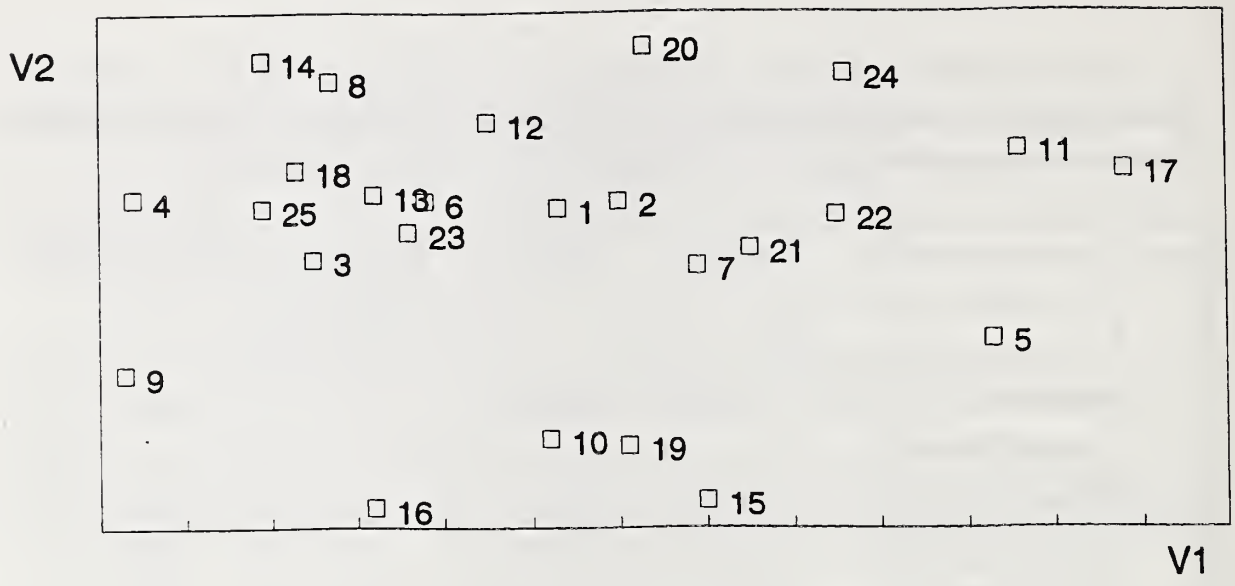


Figure 1. Configuration of 25 genotypes of soybean on V1/V2 chart

References:

Broich, S. and R.G. Palmer. 1980. A cluster analysis of wild and domesticated soybean phenotypes. Euphytica 29:23-32.

Brown, D. M. and L.J. Chapman. 1960. Soybean ecology II. Development temperature-moisture relationship from field studies. Agron. J. 52: 496-499.

Hague, M.F. 1977. Soybean breeding in India. Soybean Genet. Newsl. 4: 62-66.

Holmberg, S. A. 1973. Soybeans for cool temperate climates. Agri Hortique Genetica XXXI: 1-20.

Jaranowski, J., H. Skorupska, G. Konieczny, A. Muszynski, and L. Torz. 1983. Charakterystyka zmiennosci wazniejszych pod wzgledem gospodarczym cech uzytkowych soi (Characteristics of variability of some economically important useful features of soybean). Zesz. Probl. Post. Nauk Roln. 253:7-24.

Konowa, L., P. Marinowa, J. Prodawow, B. Macow and L. Rainowa. 1975. Wplyw warunkow ekologicznych na wzrost, rozwoj i plonowanie soi. Biuletyn IHAR 5-6: 178-188.

Mackiewicz, Z.. 1959. Krytyczne okresy wilgotnosciowe w rozwoju soi. Hodowla Roslin Aklimatyzacja i. Nasiennictwo 3-4: 507-514.

Muszynski, A. and J. Jaranowski. 1983. Interrelationships between traits as a basis for estimation of selection value in soybean (Glycine max (L.) Merrill). Genetica Polonica 24-1:51-58.

Shwe, U.K., B.R. Murty, H.B. Singh, and N.B. Rao. 1972. Genetic divergence in recent elite strains of soybean and groundnut in India. Indian J. Genet. 32:285-297.

Grzegorz Konieczny
Adam Muszynski
Jerzy Nawracala

AGRICULTURAL UNIVERSITY
Department of Genetics and Plant Breeding
60-625 Poznan ul. Wojska Polskiego 71 C, POLAND

4) Breeding evaluation of *G. max* X *G. soja* crosses

Soybean breeding in Poland is carried out at the very high north latitude (50° N). The most of available soybean variability is not useful in Polish climate. Looking for new germplasm the wild species *Glycine soja* was chosen as a potential component for crossing. IG. *soa* grows in the north part of China and in the part of Siberia (Russia) at the latitude similar to Poland (Palmer and Newhouse, 1984; He Zhihong, 1989). Therefore, genotypes from this area may be photoneutral and adapted to the Polish environment. Additionally, *G. soja* is a source of genes determining high protein contents (Kaizuma and Fukui, 1974; Yang *at al.*, 1993).

Plant Material and Methods: Two of our own very early lines of *G. max* and one genotype of *G. soja* originated from Siberia, were chosen for crossing (Tables 1 and 2). Crosses were made in 1987. The F_1 plants were grown in a greenhouse. The F_2 (379 plants) and F_3 (2686 plants) generations and their parents were planted in the field in the spacing of 40 cm between rows and 10 cm between plants in row. All plants were harvested. The F_4, F_5 and F_6 generations were planted in 1993 including checks (Polish cultivars Progres and Nawiko). Individual plants performing better than checks were selected.

Results and Discussion: From interspecies crosses we obtained 21 F_1 plants in following two cross combinations: 104 x 11 and 2349 x 11. All F_1 plants resembled the phenotype of the wild male parent. Seeds harvested from F_1 plants were intermediate in the one hundred seed weight (9.2 g) and had yellow, female parent-like, seed coat color. Germination of F_2 seeds was very poor (50%) because of seed hardness and soil drought. In the population of F_2 plants the wild, *G. max,* and intermediate phenotypes were observed. A tremendous scope of segregation occurred. Most plants matured late, but the earliest plants were harvested 14 days before the earliest 104 line. Range of plant height was very wide (Table 1). The most of plants were higher than *G. soja* parent. Only those plants which produced secondary branches showed the highest number of branches per plant. There is strong correlation between pod and seed number per plant in soybean, thus the range and frequency distribution of these two characteristics were similar. About 30% of F_2 population produced more pods and seeds than the best parent. Interesting segregants which produced seeds smaller than *G. soja* appeared. The plants yielding more than 30.0 g seeds were found in 10% of F_2 population.

In F_3 generation the tremendous scope of segregation was found, too. Ranges of

characteristics (Table 2) were similar to the ranges in F_2 generation (Table 1). The parameters of seed yield components showed greater values than plants in F_2 generation because weather conditions in 1990 were favorable for soybean. Frequency distributions of all characteristics were similar to those in F_2 generation.

In F_4 and F_5 generations the selection of individual plants was carried out. Finally, in F_6 generation, several valuable lines were selected:

- early lines: 14 days earlier than line no. 104
- very tall lines: plant over 100 cm tall
- "bushy" type lines: plants with 17 branches including secondary branches
- small seeded lines: a hundred seed weight of 5.0 g
- high yielding lines

Conclusions:

1. Variability in F_2 and F_3 generation of *G. max* X *G. soja* was very great and greater than that found in intraspecific *G. max* crosses.
2. In all F_2 - F_6 generations transgressive segregants (better than parents) in important agronomic characteristics were found.
3. Crossing between *G. max* and *G. soja* in Polish climate conditions gave the new potential to soybean breeding.

Table 1. Performances of some characteristics of parents and F_2 generation of G. max X G. soja crosses (Dlon, 1989).

Characteristics	Parents			Crossings	
	G. max		G. soja	F_2 generation	
	104 X	2349 X	11 X	104 X 11 range	2349 X 11 range
Growth period (days)	124.7	125.7	165.6	111.0-167.0	112.0 -167.0
Plant height (cm)	40.4	41.2	57.4	12.0-145.0	10.0-145.0
Branches / plant	3.3	4.5	7.0	1.0-18.0	2.0-18.0
Pods / plant	39.9	45.1	71.4	4.0-320.0	10.0-302.0
Seeds / plant	63.5	67.4	111.4	7.0-560.0	18.0-521.0
100 seed weight (g)	14.1	17.6	6.5	3.0-19.1	3.6-18.3
Yield /plant (g)	8.9	11.9	7.2	0.5-65.0	2.0-60.0

Table 2. Performances of some characteristics of parents and F_3 generation of G. max X G. soja crosses (Dlon, 1990).

Characteristics	Parents			Crossings	
	G. max		G. soja	F_3 generation	
	104 X	2349 X	11 X	104 X 11 range	2349 X 11 range
Growth period (days)	129.4	135.6	152.2	111.0-166.0	121.0-165.0
Plant height (cm)	35.0	44.0	68.7	14.0-145.0	16.0-140.0
Branches / plant	4.8	5.7	6.5	1.0-13.0	1.0-17.0
Pods / plant	67.2	79.1	107.3	12.0-373.0	10.0-419.0
Seeds / plant	121.3	140.4	198.5	12.0-891.0	16.0-767.0
100 seed weight (g)	16.1	17.2	5.8	3.0-19.1	3.3-20.7
Yield / plant	20.1	23.5	12.2	1.7-85.2	1.0-72.2

References:

He Zhihong. 1989. Ecological types of chemical composition of *G. soja* and their ecogeographical distribution. Soybean Genet. Newsl. Vol. 16:43-48.

Kaizuma, N. and J. Fukui. 1974. Specific characteristics and varietal differences for seed protein percentage and sulfur containing amino acid contents in Japanese wild soybean (*Glycine soja*), and its significance on the soybean (*Glycine max*) breeding program. Japan. J. Breed. Vol. 24-2:65-72

Palmer, R.G. and K.F. Newhouse. 1984. Evaluation of *Glycine soja* from The People's Republic of China and the USSR. Soybean Genet. Newsl. 11:105-111.

Yang, G. H. Zheng, C. Han and F. Ji. 1993. A preliminary study on protein and oil contents of wild soybean (*G. soja*) in Jilin province. Soybean Genet. Newsl. 20:35-38.

Jerzy Nawracala

Grzegorz Konieczny

FEDERAL AGRICULTURAL RESEARCH STATION (RAC)
P.O. Box 254, CH-1260
Nyon, Switzerland

Description of two flowering types and F_2 segregration in relation to pubescence color

Introduction: The reported work was initiated following repeated observation that gray pubescent genotypes showed reduced yield stability and that this behavior may be due to a particular blooming pattern. Lateral racemes have been described to flower some days after the central raceme of the same node (Gai et al., 1984; Vidal and Hanafiah, 1985; Roumet et al., 1990). We found, however, that while the interval between central and lateral blooming ranges mostly between five and 15 days, in some genotypes lateral blooming on a given node starts within one to five days after the beginning of central blooming. Observations on our breeding material revealed that this "synchronous blooming" mostly occurs on genotypes with gray pubescence color, while "asynchronous blooming" mostly occurs on genotypes with tawny pubescence color. This led us to verify whether a linkage exists between the synchronism of blooming and pubescence color (T/t) belonging to linkage group 1. To describe the phenotypical effect of this trait and acquire more knowledge about its inheritance F_1 descendants and F_2 hybrid populations of (gray-synchronous x tawny-asynchronous) crosses were studied.

Materials and Methods: The duration of blooming for both central and lateral racemes of each node was recorded on 'Evans' (MG 0, gray-sychronous), 'Ceresia' (MG 00, tawny-asynchronous) and the F_1 [Evans x Ceresia]. These observations were conducted in growth chambers (16 hours of light; temperatures: 25° C/18° C).

The F_2 hybrid populations of the following (gray-synchronous x tawny-asynchronous) crosses were studied: [CH20959 x Silvia], [CH20959 x Ceresia] and [CH20959 x X1997B-13-8-B]. CH20959 is a homozygous breeding line from the cross [OX611 x (Maple Presto x Evans)]. 'Silvia' and 'Ceresia' are two Swiss varieties (MG 00). X1997B-13-8-B is a photoperiod insensitive near-isoline of 'Maple Arrow' (e3e3, MG 000,Saindon, et al., 1989) kindly supplied to us by H.D. Voldeng, Ottawa, Ontario. The blooming habit was determined visually at the R_2 stage.

To gain knowledge about the origin of synchronous blooming, its presence or absence was determined on the following progenitors of 'Evans': 'A.K.' (Harrow), 'Mandarin', 'Mukden' and 'Richland'. These were kindly supplied to us by the USDA Soybean Germplasm Collection, Urbana, Illinois.

Results and Discussion: Figure 1 illustrates the duration of blooming on central and lateral racemes of the first seven purely reproductive nodes from the anthesis of the first flower to the anthesis of the last flower. The blooming habit of the F_1 is that of the parent 'Ceresia'.

The phenotypical segregation of pubescence color and synchronism of blooming within the three F_2 hybrid populations is given in Table 1. While the crosses show good agreement with the 3:1 segregation expected for pubescence color, the ratios observed for both traits strongly deviate from the expected 9:3:3:1 ratio for the two independent genes. Table 2 gives the comparison of Chi-square to alternative F_2 ratios segregating in synchronous and asynchronous blooming. The Chi-square values observed permit the rejection at 0.05 level of the 3:1, 9:7, 15:1 and 13:3 models. The 11:5 model, however, can not be rejected, suggesting that synchronism is controlled by two genes with a modified dominance similar to that described for cotton by Fuchs et al., (1972). If the 11:5 model is true and the three genes (one for pubescence color and two for synchronism) are independent, the expected segregating ratio would be 33:15:11:5 (Table 1). A comparison with the observed segregation of the four phenotypes shows an important deficit in non-parental phenotypes and the Chi-square comparisons require the rejection of the independence hypothesis at the 0.01 level. This suggests that one of the two genes controlling synchronism is linked to pubescence color. F_3 tests have to undertaken to confirm or refute these hypotheses.

The observation of the progenitors of 'Evans' (Table 3) showed that this variety traces back to both gray-synchronous and gray-asynchronous cultivars. Lohnes and Bernard (1991) state that of 41 Group 000-0 US/Canadian cultivars 31 have 'A.K.' and 24 have 'Mukden' in their ancestry. Both these cultivars being gray-synchronous, we expect the trait to be widespread.

Implications: Experiments including the removal of flowers (Schori et al., 1993) and field observations under cold stress conditions during flowering (Gass et al., 1993), suggest that synchronous blooming increases the contribution of lateral flowers to the overall yield under favorable conditions. However, the simultaneous presence of central and lateral flowers under

stressful conditions prevents the latter from avoiding the stress and thus playing the important compensatory role they have on asynchronous genotypes. This trait may be of most importance in high latitudes (cool climates) and on early material where flower number on central racemes is relatively low. The low frequency of non-parental phenotypes (12.5%) together with the fact that genitors in our breeding program are mostly of the asynchronous/tawny or of the synchronous/gray type explain why we wrongly associated gray pubescence with yield instability.

References:

Fuchs, J.A., J.D. Smith and L.S. Bird. 1972. Genetic basis for an 11:5 dihybrid ratio observed on Gossypium hirsutum. J. Hered. 63:300-303.

Gai, J., R.G. Palmer and W.R. Fehr. 1984. Bloom and pod set in determinate and indeterminate soybeans grown in China. Agron. J. 76:979-984.

Gass, T., A. Schori, A. Fossati, A. Soldati and P. Stamp. 1993. Some components contributing to tolerance of soybean [Glycine max (L.) Merr] to low temperature stress during flowering. In COST 814 Workshop proceedings, Aberysthwyth, U.K.

Lohnes, D.G. and R.L. Bernard. 1991. Ancestry of US/Canadian commercial cultivars developed by public institutions. Soybean Genet. Newsl. 18:243-255.

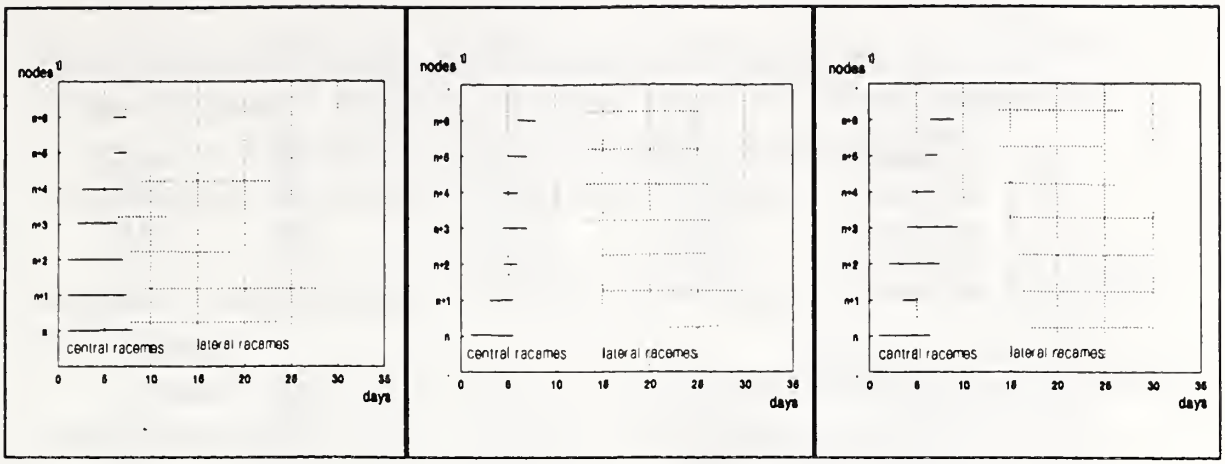
Roumet, P., T.O. Hanafiah and A. Vidal. 1990. Etude de la croissance et du developpement de lignees quasi isgeniques pour leur type de croissance. In: Proceedings of the CETIOM Symposium EUROSOYA, Strasbourg, France.

Saindon, G., H.D. Voldeng, W.D. Beversdorf and R.I. Buzzell. 1989. Genetic control of long daylength response in soybean. Crop. Sci. 29:1436-1439.

Schori, A., A. Fossati, A. Soldati and P. Stamp. 1993. Cold tolerance in soybean in relation to flowering habit, pod set and compensation for lost reproductive organs. Eur. J. Agron. 2:173-178.

Vidal, A. and T.O. Hanafiah. 1995. Influence du type de croissance sur la morphologi et la floraison du soja [Glycine max (L.) Merr.] Eurosoya 3:62-70.

Arnold Schori
Thomas Gass



a) Evans (gray) b) Ceresia (tawny) c) F1[Evans x Ceresia] (tawny)

[1)] n = first purely reproductive node

Figure 1. Duration of blooming on central and lateral racemes of the seven first purely reproductive nodes from anthesis of the first flower to anthesis of the last flower. Genotypes : a) Evans, b) Ceresia and c) F1 [Evans x Ceresia].

Table 1. Phenotypical segregation of pubescence color and blooming synchronism and percentage of non-parental phenotypes in three F2 hybrid populations. $\chi 2$ fits to 9:3:3:1 and 33:15:11:5 ratios.

F2[gray/syn.x tawny/asyn.]		tawny-asyn.	tawny-syn.	gray-asyn.	gray-syn.	total	$\chi 2$	P	% of non-parental phenotypes
[CH20959 x Silvia]									
observed:		**149**	**19**	**8**	**58**	**234**			**11.5**
9:3:3:1	expected:	131.63	43.88	43.88	14.63	234	174.32	<.01	37.5
33:15:11:5	expected:	120.66	54.84	40.22	18.28	234	142.20	<.01	40.6
[CH20959 x Ceresia]									
observed:		**152**	**28**	**8**	**56**	**244**			**14.8**
9:3:3:1	expected:	137.25	45.75	45.75	15.25	244	148.52	<.01	37.5
33:15:11:5	expected:	125.81	57.19	41.94	19.06	244	119.41	<.01	40.6
[CH20959 x M.Arrow e3e3e4e4]									
observed		**172**	**18**	**11**	**56**	**257**			**11.3**
9:3:3:1	expected:	144.56	48.19	48.19	16.06	257	152.15	<.01	37.5
33:15:11:5	expected:	132.52	60.23	44.17	20.08	257	130.54	<.01	40.6
total									
observed:		**473**	**65**	**27**	**170**	**735**			**12.5**
9:3:3:1	expected:	413.44	137.81	137.81	45.94	735	471.17	<.01	37.5
33:15:11:5	expected:	378.98	172.27	126.33	57.42	735	388.96	<.01	40.6

Table 2. Comparison of $\chi 2$ fits to alternative F2 ratios in populations segregating for synchronous and asynchronous blooming.

Hypothesis		asyn.	syn.	total	$\chi 2$	P
F2 [CH20959 x Silvia]						
	observed	**157**	**77**	**234**		
3:1	expected	175.50	58.5	234	7.80	<0.01
9:7	expected	131.62	102.38	234	11.18	<0.01
15:1	expected	219.37	14.63	234	283.67	<0.01
13:3	expected	190.12	43.88	234	30.77	<0.01
11:5	expected	160.90	73.10	234	0.30	0.5-0.7*
F2 [CH20959 x Ceresia]						
	observed	**160**	**84**	**244**		
3:1	expected	183.00	61.00	244	11.56	<0.01
9:7	expected	137.25	106.75	244	8.62	<0.01
15:1	expected	228.75	15.25	244	330.6	<0.01
13:3	expected	198.25	45.75	244	39.36	<0.01
11:5	expected	167.75	76.25	244	1.13	0.2-0.3*
F2 [CH20959 x M.Arrow e4]						
	observed	**183**	**74**	**257**		
3:1	expected	192.75	64.25	257	1.97	0.1-0.2*
9:7	expected	144.56	112.44	257	23.36	<0.01
15:1	expected	240.93	16.06	257	222.95	<0.01
13:3	expected	208.81	48.19	257	17.05	<0.01
11:5	expected	176.70	80.30	257	0.72	0.2-0.3*
Total[1)]						
	observed	**500**	**235**	**735**		
3:1	expected	551.25	183.75	735	19.06	<0.01
9:7	expected	413.44	321.56	735	41.42	<0.01
15:1	expected	689.06	45.94	735	829.93	<0.01
13:3	expected	597.19	137.81	735	84.36	<0.01
11:5	expected	505.30	229.70	735	0.18	0.7-0.9*

[1)] Homogeneity of the crosses : $\chi 2$ = 5.99, P: 0.3-0.5,*
* The hypothesis cannot be rejected at 0.05 level.

Table 3. Blooming habit of the progenitors[1)] of 'Evans' (gray-synchronous)

	pubescence color	blooming habit
Cultivar:		
A.K.(Harrow)	gray	syn.
Mandarin	gray	asyn.
Mukden	gray	syn.
Richland	gray	asyn.
No. 171[2)]	—	—

1) Ancestry according to Lohnes and Bernard (1991)
2) No. 171 was not studied

[1] Dept. of Plant Pathology, UNIV. OF MINNESOTA, St. Paul, MN
[2] Dept. of Agronomy, IOWA STATE UNIV., Ames, IA
[3] Horticultural Sciences Dept., UNIV. OF FLORIDA, Gainesville, FL
[4] USDA/ARS & Dept. of Agronomy, IOWA STATE UNIV., Ames, IA

Genome Conservation between *Glycine* and Legume Relatives Detected with DNA Markers

Abstract: To detect synteny among the genomes of three legume genera (*Vigna*, *Phaseolus*, *Glycine*), we compared the linkage order of 173 RFLPs common to at least two of the genera. Extensive linkage conservation was observed between *Vigna* and *Phaseolus*, with some linkage groups conserved virtually intact. Nearly every *Vigna* linkage group corresponded to only one or two *Phaseolus* group(s). The level of genome conservation between the genomes of *Vigna* and *Phaseolus* and that of *Glycine* was lower, but still substantial.

In some cases, linkage blocks up to 35 cM in length were conserved among all three genera. On some conserved linkage groups, rearrangements could be detected, while in other cases, duplicated blocks were observed, especially in *Glycine*. Based on these results, it is likely that linkage mapping data and database resources can be shared among research efforts in different legume species.

Introduction: Characterizing genome evolution among related plant taxa is a powerful application of DNA marker technology. By mapping a common set of markers in genetically isolated groups, changes in chromosome organization can easily be discerned. Recently, this strategy has been used to demonstrate extensive genome conservation among different *Gramineae* genera, including *Oryza*, *Sorghum*, and *Zea*, (Ahn and Tanksley, 1993; Whitkus *et al.* 1992) as well as among genera in the *Solanaceae* (Prince and Tanksley, 1992; Bonierbale *et al.* 1988) and the *Cruciferae* (McGrath and Quiros, 1991). DNA markers have also been used to examine genome conservation between *Pisum* and *Lens*. (Weeden *et al.* 1992), and *V. radiata* and *V. unguiculata* (Menancio-Hautea *et al.* 1993).

A striking result of all these research efforts has been the remarkable degree to which genomic blocks are maintained among plant genera in a single family. This includes taxa that are considered to be quite distant evolutionarily.

Genome conservation has been uncovered even against a backdrop of genomic duplication.

Materials and Methods: **Parental Genotypes.** Three mapping populations were used to analyze a common set of RFLP markers. One F2 population (58 individuals) was derived from a cross between *V. unguiculata* (VC3890) and subspecies, *sublobata* (TC1966). A second F2 population (60 individuals) was derived from a cross between *G. max* (A81-356022) and *G. soja* (PI 668916). A third (backcross) population of 68 individuals was derived from a cross between *P. vulgaris* line XR-235-1-1 (Mesoamerican) and 'Calima' (Andean).

RFLP Mapping. A total of 172 RFLPs were analyzed in the three populations, with 76 common between *Vigna* and *Glycine*, 87 common between *Vigna* and *Phaseolus*, and 61 common between *Glycine* and *Phaseolus*; 28 RFLPs were common to all three. The most likely marker orders (LOD 2.0) were determined with Mapmaker-II. Conserved genomic regions were uncovered by identifying blocks of linked markers common among two or more genera.

Results: There are several instances of conserved linkage blocks among all three genera. Essentially all linkage groups in the *Vigna* and *Phaseolus* genomes are common between the two. Many conserved regions extend the entire length of a linkage group. By contrast, common linkage blocks are shorter and more scattered in *Glycine*. For example, the longest conserved linkage block (already identified) between *Vigna* and *Glycine* is only 35 cM in length and there are several cases where contiguous markers in *Vigna* and *Phaseolus* are apparently unlinked in *Glycine*.

In detailed comparisons, a clear example of a balanced translocation can be observed between *Vigna* groups 1 and 9 and *Phaseolus* groups F and H (Fig. 1). In a side-by-side comparison of all three genera, *Vigna* group 7 and *Phaseolus* group G are nearly identical. Both contain blocks from *Glycine* J and L plus shorter segments from four other *Glycine* linkage groups (Fig. 2).

Vigna groups 5 and 8 combine to form *Phaseolus* group K, and *Vigna* 5 and the bottom of *Phaseolus* K are highly conserved with *Glycine* C (Fig. 3). By contrast, *Vigna* 8 and the top portion of *Phaseolus* K correspond to segments from as many as eight different *Glycine* linkage groups.

Finally, it appears that some conserved segments among the three genera are duplicated in the *Glycine* genome. For example, both the top of *Phaseolus* G and the bottom of *Phaseolus* J correspond to genomic blocks that

are duplicated in *Glycine*. This resembles the kind of genomic relationship that has previously been observed between *Oryza* and *Zea*.

Figure: Detailed comparisons of linkage groups/blocks that are conserved among *Vigna, Phaseolus*, and *Glycine*.

Panel 1 shows a balanced translocation between linkage groups 1 and 9 of *Vigna* and linkage groups F and H of *Phaseolus*.

Panel 2 shows linkage groups 7 of *Vigna* and G of *Phaseolus*, which are conserved essentially intact. Beside the *Phaseolus* and *Vigna* groups, the corresponding *Glycine* blocks are shown.

Panel 3 shows linkage groups 8 and 5 of *Vigna*, which together correspond to *Phaseolus* group K. Beside the *Phaseolus* and *Vigna* groups, the conserved *Glycine* blocks for each are shown, including linkage block C, a region that is largely conserved among all three genera.

Distances are based on centimorgans as measured in the mapping population of the corresponding genus. (**Note**: The letters used to designate *Phaseolus* linkage groups have no relationship to those designating *Glycine* linkage groups).

References:

Ahn S. and S.D. Tanksley (1993) Comparative linkage maps of the rice and maize genomes. Proc. Natl. Acad. Sci. USA 90: 7980 - 7984.

Bonierbale M.W., R. L. Plaisted, and S.D. Tanksley (1988) FRLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095-1103.

Menancio-Hautea D., C.A. Fatokun , L. Kumar , D. Danesh , N.D. Young (1993) Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walpers) using RFLP mapping data. Theoretical and Applied Genetics 86:797-810.

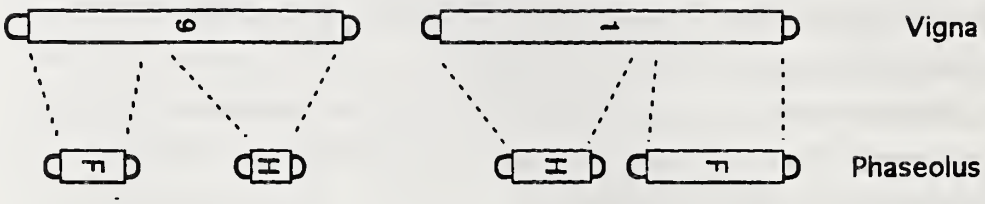
Prince J.P., and S.D. Tanksley (1992) Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. Genome 36: 404-417.

Weeden N.F., F.J. Muehlbauer , and G. Ladizinsky (1992) Extensive conservation of linkage relationships between pea and lentil genetic maps. The Journal of Heredity 83: 123-129.

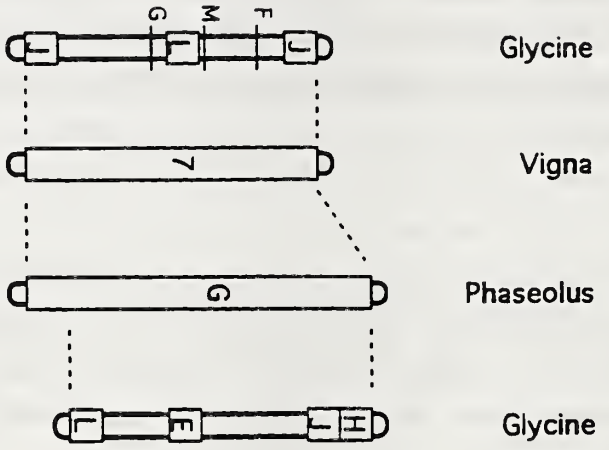
Whitkus R., J. Doebley , and M. Lee (1992) Comparative genome mapping of sorghum and maize. Genetics 132: 1119-1130.

S. Boutin[1] N. Young[1]
T. Olson[2] R. Shoemaker[4]
Z.-H. Yu[3] E. Vallejos[3]

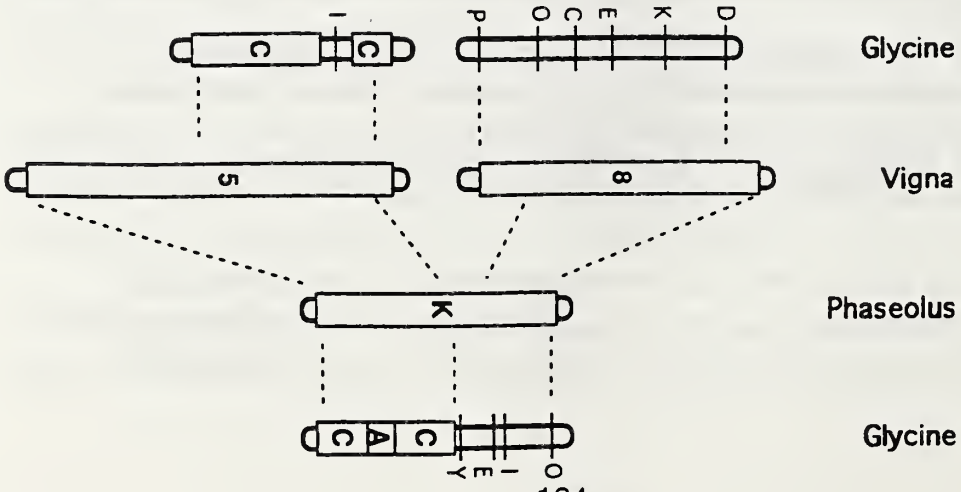
1


9
1
Vigna
F
H
H
F
Phaseolus

2


G
M
F
Glycine
Vigna
Phaseolus
Glycine

3


I
P
O
C
E
K
D
Glycine
Vigna
Phaseolus
Glycine
Y
E
I
O

USDA, ARS FIELD CROPS RESEARCH UNIT
IOWA STATE UNIVERSITY
Department of Zoology/Genetics &
Department of Agronomy
Ames, Iowa 50011

Screening the USDA germplasm collection for malate dehydrogenase variants

Major efforts have been made to map morphological, biochemical, and disease resistance loci in soybean using traditional and molecular techniques. We have been interested in studying malate dehydrogenase (MDH) in soybean. Cardy and Beversdorf (1984) reported two MDH variants. Palmer et al. (1992) reported that the Rxp locus and a MDH variant were linked, based on 650 F_2 plants from reciprocal crosses of 'Clark 63' x PI 437477B. A percentage recombination of 15.18 ± 3.81 was obtained. Plants with the rxp rxp genotype are resistant to bacterial pustule caused by Xanthomonas campestris pv. glycines.

Hedges and Palmer (1992) tested three chlorophyll-deficient mutants (T323, T324, and T325) derived from the progeny of independent germinal revertants from the w4-mutable line (T322) for MDH. Electrophoretic analyses indicated that these lines lacked two of three mitochondrial malate dehydrogenase isozymes. The absence of two MDH bands was conditioned by a recessive allele at a locus designated Mdh1.

Seeds were analyzed for MDH using starch gel electrophoresis technique (Hedges et al. 1992). Data are reported in Table 1. No malate dehydrogenase nulls were found. We found 47 lines with the MDH-A and 58 lines with MDH-B zymogram patterns.

Table 1. Evaluation of soybean germplasm for malate dehydrogenase

Cultivar, Genetic Type Collection number, or accession number

MDH-A	MDH-B
Calland	Beeson
Century	BSR 101
Corsoy 79	Chippewa
Cutler	Chippewa - rxp
Cutler 71	Clark
Hardin	Clark 63
Harosoy	Clark-i r-m
Harosoy-rxp	CNS
Manchu	Elgin
Mandarin	Harrow
Mandarin (Ottawa)	Jefferson
Richland	Lincoln
Swift	Minsoy
Williams 82	Old Dominion
Wye	Pine Dell Perfection
T146	PI 91073
	PI171443
PI 79583	PI179826
PI 82308	PI243536
PI 84631	PI398329
PI 86026	PI398431
PI 86029	PI398442
PI 86145	PI398450
PI 88492	PI398549
PI 91729	PI398676
PI 96321	PI398920
PI179823	PI399004
PI398203	PI399013
PI398349	PI399072
PI398355	PI408023
PI398357	PI408093
PI398410	PI408111
PI398487	PI408114
PI398551	PI408126A
PI408046	PI408126B
PI416817	PI408149
PI417064	PI408194
PI417343	PI408273
PI417344	PI408310A
PI423769A	PI408310B
PI424174	PI408325
PI424561	PI417168
PI437332	PI417482
PI437477B	PI423735

Table 1. (cont.) Evaluation of soybean germplasm for malate dehydrogenase

Cultivar, Genetic Type Collection number, or accession number

MDH-A	MDH-B
PI438299	PI423757
PI458129	PI424158
PI479729	PI424160
PI507148	PI424232A
	PI424232B
	PI424274A
	PI424274B
	PI424481A
	PI424481B
	PI424600
	PI424604
	PI458034
	PI458098
	PI458302

References:

Cardy, B. J. and W. D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes in soybean [Glycine max (L.) Merr.]. Dep. Crop Sci. Tech. Bull. 119/8401. University of Guelph, Ontario, Canada.

Hedges, B. R. and R. G. Palmer. 1992. Inheritance of malate dehydrogenase nulls in soybean. Biochem. Genet. 30:491-502.

Hedges, B. R., R. G. Palmer, and L. A. Amberger. 1992. Electrophoretic analysis of soybean seed protein. In H. F. Linskens and J. F. Jackson (eds.) pp. 143-158. Modern Methods of Plant Analysis. Springer-Verlag, Berlin.

Palmer, R. G., S. M. Lim, and B. R. Hedges. 1992. Testing for linkage between the Rxp locus and nine isozyme loci in soybean. Crop Sci. 32:681-683.

T. Couch - USDA
R. G. Palmer- USDA

USDA/ARS
Beltsville, MD 20705
and
PA, VA, MD, and DE experiment stations

Relative Performance of Soybean Cultivars, Ancestral Cultivars, and Plant Introductions.[1/]

Abstract: A total of 113 soybean [Glycine max (L.) Merr.] plant introductions (PI's) representing maturity groups II through V were evaluated. The PI's were assigned to four experiments corresponding to maturity group. In addition to adapted genotypes, ancestral cultivars were included in experiments when their maturity classification was appropriate. Each experiment was grown in a minimum of five environments. In maturity groups II and III the mean yield over environments of the more recently developed or released genotypes was about 16% greater than the ancestral cultivars, indicating the significant agronomic improvements resulting from soybean breeding. In the same experiments, the mean yields of the ancestral cultivars and the PI's were similar, suggesting that the ancestral cultivars possess a yield potential which is no greater than many PI's. Five PI's produced mean seed yields which were significantly higher than the mean ancestral cultivar yields and numerous other PI's had mean yields which numerically exceeded the ancestral cutivars. Twelve of these PI's were received in the U. S. at least 12 years before the release of the first U.S. cultivars developed from planned hybridizations, and therefore would have been available for use in early soybean breeding programs. Of these 12 PI's , only 3 had lodging scores which were numerically equal or superior to the ancestral cultivar mean. Thus, many productive PI's were probably overlooked in early soybean breeding programs because of the immediate necessity of developing soybean genotypes with superior lodging resistance. To create unique and useful genetic diversity and maintain the long-term genetic improvement in soybean performance, we suggest the development of soybean populations using these and similarly productive PI germplasm. The exclusion of adapted genotypes may be desirable because selection in populations containing a significant proportion of adapted germplasm may tend to rapidly eliminate genetic diversity in favor of genotypes which are genetically similar to adapted parents.

Additional index words: [Glycine max (L.) Merr.] Genetic divestity, Germplasm diversification.

In 1939 the principal soybean [Glycine max (L.) Merr.] cultivars grown in the North Central U.S. were 'Dunfield', 'Illini', 'Macoupin', 'Manchu', 'Mandarin', 'Mandell', 'Mukden', 'Richland', and 'Scioto' (Hartwig, 1973). With the Exception of Macoupin, whose origin is unknown, these cutivars are either introductions or selections from introductions originating in northeast China (Hymowitz et. al., 1977). The release of U.S. soybean cultivars developed from planned hybridizations began in the 1940's. By the early 1960's the ancestral cultivars Mandarin, Manchu, Richland, Mukden, 'A.K.' and Dunfield, all from northeast China, formed the exclusive background for cultivars in maturity groups 00 to IV grown on 95 percent of the northern U. S. soybean hectarage (Johnson and Bernard, 1963). As soybean breeding has progressed through cycles of hybridization and selection, the use of relatively few adapted parental strains without the addition of new germplasm has resulted in the loss of a substantial portion of the genetic variation originally present in northern cultivars (St. Martin, 1982; Delannay et. al. 1983).

Limited genetic variation among commercial cultivars of a crop species is indicative of genetic vulnerability to a new pest (National Academy of Sciences, 1971) and also could be detrimental to further genetic improvement in productivity if only these cultivars are used as parents in breeding programs. However, careful attention of soybean workers has allowed the identification and incorporation of sources of resistance to new pests or variants of established pests, thereby minimizing soybean production losses. Furthermore, a number of recent studies indicated continuous long-term genetic improvements in soybean yield potential (Luedders, 1977; Boerma, 1979; Wilcox et. al., 1979; Specht and Williams, 1983) suggesting that genetic improvements have not been curtailed by declining genetic variability. Nevertheless, the continued use by soybean breeders of closely related elite genotypes seems likely to limit genetic gains in the foreseeable future.

Schoener and Fehr (1979) suggested that increased genetic diversity be attained by using exotic germplasm. Soybean plant introductions with good yield potential could be used along with adapted genotypes in the development of breeding populations for long term germplasm development programs. The use of exotic soybean germplasm in yield improvement was reported by Kenworthy and Brim (1979). They completed three cycles of recurrent selection for yield in a population developed by intermating backcross progeny of one adapted line with nine plant introductions. A composite of elite lines from cycle three out yielded the adapted parent and a composite of lines from the base population by 20 and 16% respectively.

The results support the suggestion that exotic soybean germplasm may be useful in the development of populations serving as sources of highly productive genotypes.

The plant breeder interested in synthesizing soybean populations using plant introductions with good yield potential, as suggested by Schoener and Fehr (1979), would have difficulty identifying such plant introductions because there is little published information regarding the evaluation of exotic soybean germplasm over a range of environments. We are also unaware of studies which compare soybean plant introductions with the important ancestral cultivars which were originally used commercially and later served as parents in early soybean breeding programs. Our objectives in the current work were therefore, (a) to evaluate the agronomic potential of a large number of soybean plant introductions and (b) to compare these introductions with the important ancestral cultivars and with a group of adapted soybean cultivars.

Materials and Methods: A total of 556 maturity group II, 646 maturity group III, and 1,294 maturity group IV soybean plant introductions (PI's) were obtained from the USDA germplasm collection courtesy of Dr. R.L. Bernard (USDA, ARS, Agronomy Department, University of Illinois, Urbana, IL). In addition, 297 maturity group V PI's were obtained from the USDA germplasm collection, courtesy of Dr. E. E. Hartwig (USDA, ARS, Delta Branch Experiment Station, Stoneville, MS).

In 1976, the maturity group II and III PI's were planted in the field at Blacksburg, VA, and inoculated with peanut mottle virus. Genotypes with apparent resistance to the virus which also demonstrated minimally acceptable lodging and shattering resistance, visual seed quality, and vigorous growth were evaluated in an unreplicated yield trial at Blacksburg, VA, in 1977. In 1978, 48 maturity group II and 72 maturity group III PI's were further evaluated for yield in unreplicated trials at Adelphia, NJ; Landisville, PA; Beltsville, MD; and Orange, VA. Based upon these trials and the agronomic and seed quality characteristics, 15 maturity group II and 32 group III PI's were selected for further evaluation from 1979 to 1981.

In 1977, the maturity group IV and V PI's were grown in 1.5 m rows at Queenstown, MD, and in hill plots at Beltsville, MD, and Warsaw, VA. Those genotypes with minimally acceptable lodging and shattering resistance, visual seed quality , and vigorous growth were grown in unreplicated yield trials at Beltsville, MD; Georgetown, DE; and Warsaw, VA, in 1978. Agronomic and seed quality characteristics observed in these experiments were used to select 54 maturity group IV and 12 maturity group V PI's for further evaluation in 1979 and 1980.

Based upon our observations of maturity from 1976 to 1978, the selected PI's were assigned to four experiments corresponding to maturity groups II, III, IV, and V

(Table 1). Our observations did not always correspond to the maturity classification assigned in the USDA germplasm collection. Each experiment also included a number of adapted genotypes. Depending upon their maturity classification, seven different ancestral cultivars were entered in Experiments II to IV (Table 1). However, Mandarin (Ottawa), which is classified as maturity group 0, was included in Experiment II and A.K. (Harrow)) and Dunfield, both classified as maturity group III, were placed in Experiment III and Experiment IV. Simple lattice experimental designs were used for Experiments II, III, and IV. Experiment V was a randomized complete block with two replications (Table 2). Each experiment was grown in a minimum of five environments. A plot consisted of four rows 3.6 m long with 0.76 m between rows. The planting rate was 26 seeds/m of row. At maturity the two center rows were trimmed to 2.5 m and harvested to obtain an estimate of seed yield. Other characters evaluated included time of maturity when 95% of the pods reached their mature color, height from the soil surface to the terminal bud at maturity, lodging score which ranged from 1.0 (all plants erect) to 5.0 (all plants prostrate), and visual seed quality which ranged from 1.0 (healthy seed, intact seed coat) to 5.0 (diseased seed, poor seed coat integrity).

Lattice analyses of the yield data from Experiments II, III, and IV were conducted and in those cases in which the lattice was at least 5% more efficient than a randomized complete block, adjusted treatment mean were used in the analysis over environments. A randomized complete block analysis was performed on all other traits in Experiments II, III, and IV.

In the analyses over environments the soybean genotype X environment mean square was used to test the significance of the mean square attributable to soybean genotypes.

Results and Discussion: The range in the mean yields of the trials in Experiments II, III, and V indicated the wide diversity of the test environments (Table 2). In Experiment IV, a smaller range in mean trial yields was evident. Significant differences among entries were detected for the traits measured in all trials with the exception of yield in Experiment II at Adelphia in 1979 and in Experiment V at Queenstown and Warsaw in 1979 and at Beltsville in 1980. The analyses over locations detected significant genotype effects and genotype x environment interactions in the traits measured in each of the four experiments.

Experiment II

The mean yield of the five adapted maturity group II cultivars was significantly greater than either the mean of the ancestral cultivars or the PI's (Table 3). A

comparison of the ancestral cultivars with the adapted genotypes released in the last 20 years, i.e., 'Amsoy 71', 'Beeson', and 'Harcor' indicated a 16.8% yield advantage of the adapted genotypes. Thus, the gain attributable to soybean breeding in our study is substantially less than that suggested by Luedders (1977) , Wilcox et, al. (1979), or Specht and Williams (1983). The lack of a yield advantage of the ancestral cultivars over the PI's (Table 3) suggests that the ancestral cultivars possess a yield potential which is no higher than many PI's. While no differences in the maturity of the three groups were noted, the adapted cultivars were more lodging resistant than the ancestral cultivars which, in turn had significantly better standability than the PI's. In contrast, the visual seed quality of the PI's was superior to both the adapted genotypes and the ancestral cultivars. These results indicate that lodging resistance probably was an important criterion in the selection of the soybean genotypes first released for production in the U.S., and that visual seed quality was evidently not a trait of major importance or that seed quality differences were not apparent under the conditions used in the selection of the ancestral cultivars.

The mean performance of individual lines in Experiment II demonstrates the generally superior yield potential and lodging resistance of the adapted genotypes (Table 3). Nine of the 15 PI's had mean yields numerically superior to Mukden, the highest yielding ancestral cultivar. PI 68.683, PI 384.468, and PI 96.194-1 produced yields which were significantly higher than the ancestral cultivar mean. In addition, PI 384.468 and PI 361.065B had lodging scores which were not significantly poorer than any of the adapted genotypes. Nine of the 15 PI's attained visual seed quality scores which were numerically superior to any of the adapted genotypes or ancestral cultivars.

Experiment III

As in Experiment II, the mean yield of the seven adapted checks was significantly greater than the mean ancestral cultivar or PI yield (Table 4). The adapted genotypes released or developed in the last 20 years had a yield advantage of 15.5% over the ancestral cultivars. As was true in Experiment II, this estimate of gain resulting from genetic improvement was substantially lower than that reported by other workers (Leudders, 1977; Wilcox et. al. , 1979; and Specht and Williams, 1983). The similarity in the yields of the ancestral cutivars and PI's, as was also noted in Experiment II, again suggests that the ancestral cultivars are not significantly more productive than many PI's in the soybean germplasm collection. The maturity of the ancestral cultivars was significantly earlier than the adapted genotypes or the PI's, and the range of maturity in the ancestral cultivars was quite narrow as compared to that of

the adapted genotypes and the PI's. The lodging resistance of the adapted genotypes was superior to the ancestral cultivars which in turn had significantly better standibility than the PI's. As in Experiment II, this result reflected the fact that lodging resistance probably was an important criterion in the selection of the ancestral cultivars as well as in subsequent cycles of hybridization.

The performance of individual lines in Experiment III generally demonstrated the superior yield potential and lodging resistance of the adapted genotypes (Table 4). Comparisons of individual PI and ancestral cultivar yields indicated that only two PI's produced seed yields numerically higher than the highest yielding ancestral cultivar 'AK (Harrow)'. Only PI 84.656 produced a significantly greater seed yield than the mean of the three ancestral varieties. Similar to the results in Experiment II, some PI's demonstrated lodging resistance which was not significantly inferior to the adapted genotypes. In addition, PI 196.157 and PI 398.494 had the lowest visual seed quality scores of the 42 genotypes included in Experiment III.

The PI's included in Experiment III originated from Japan, Korea, and northeastern China (Table 1), thus offering a comparison of these three germplasm sources. The mean yield of the 13 PI's from Korea was significantly higher than the mean yields of those from Japan or northeastern China. At least among this limited sample of soybean accessions, those from northeastern China were not as productive as PI's from Korea (Table 5). In addition, the PI's from northeastern China had significantly poorer visual seed quality than accessions from Japan or Korea. Early maturity is often associated with poorer seed quality, however, PI's of Chinese origin were no earlier than those from Japan or Korea (Table 5). Although the sample of PI's is very limited, these findings suggest that PI's of Korean origin may be better adapted to the Middle Atlantic states. Thus, in a soybean breeding program using exotic germplasm and aimed at the development of high yielding genotypes, particular emphasis would logically be placed upon introductions of Korean origin. As noted earlier, however, the ancestral cultivars are almost exclusively of northeastern Chinese origin. Further, the significantly poorer visual seed quality of soybean accessions from northeastern China indicates that germplasm from this source would be less desirable than materials from Japan or Korea if the production environment were particularly conducive to the diseases and seed coat abnormalities that negatively affect seed quality. As noted by Hartwig (1973) such conditions are quite common in the Middle Atlantic states where these tests were conducted.

Many plant introductions in Experiment II and III produced yields which were numerically higher and in some cases significantly higher than the mean yields of the

ancestral cultivars in the respective test. This was an unexpected result because we anticipated that the soybean accessions which were released for commercial production and later became the ancestral cultivars were probably the most productive lines available. However, this does not appear to be true. In Experiment II, PI 68.683, PI 96.194-3 and in Experiment III, PI 84.656 produced seed yields significantly higher than the respective mean ancestral cultivar yields. These introductions were received in the U.S. in 1932 or before, and thus, would have been available when the U.S. Regional Soybean Industrial Products Laboratory was established in 1936 signaling the beginning of coordinated soybean cultivar development in the U.S. In addition, the yields of another nine maturity group II and III PI's, which arrived in the U.S. in 1930 or before, numerically exceeded the mean ancestral cultivar yield. These accessions included PI 68.670-2, PI 86.122, PI 54.620, PI 80.471-1, PI 88.306, PI 87.456-2, PI 85.356, PI 88.310, and PI 88.486. Of the 12 PI's with yields numerically higher than the ancestral cultivars, only three, PI 80.471-1, PI 88..486 and PI 84.656 had lodging scores which were numerically equal or superior to the ancestral cultivar mean. Probably many productive PI's were overlooked because of the immediate necessity of developing soybean genotypes with superior lodging resistance.

Experiments IV and V

In Experiment IV a large and significant difference between the mean yield of the adapted cultivars and that of the PI's was detected (Table 6). The ten highest yielding entries in Experiment IV included the eight adapted cultivars along with PI 85.469 and PI 61.944 which ranked eighth and ninth, respectively. The PI's averaged about 3 days later in maturity than the adapted lines and also showed a much greater susceptibility to lodging. The range of the PI lodging scores indicated that certain PI's possessed a nearly prostrate growth habit. In contrast, however, the PI's as a group had significantly better seed quality than the adapted genotypes. Six PI's had visual seed quality scores which were numerically superior to any of the adapted genotypes.

In Experiment V the adapted genotypes significantly out yielded the plant introductions (Table 6), however, PI 96.983, PI 248.511, PI 339.978, and PI 324.924 were not significantly lower yielding than any of the adapted genotypes. The 12 maturity group V PI's were equal in lodging resistance to the adapted genotypes and the range of lodging scores was similar in the PI's and the adapted lines. This result is in contrast to Experiments II to IV in which the PI's consistently exhibited less lodging resistance than the adapted genotypes.

Conclusions: Our results indicate that a number of maturity group II and III accessions in the soybean germplasm collection numerically exceed, and in a few cases, significantly exceed the mean yield of the ancestral cultivars. Furthermore, in none of the four experiments we conducted did the mean yield of the highest yielding adapted genotype significantly exceed the yield of the highest yielding plant introduction. These results suggest that with careful selection, many introductions in the soybean collection may prove to be valuable sources of productive germplasm. While it is possible that such accessions may possess no desirable allelles which are not already present in current soybean germplasm, this possibility seems unlikely for two reasons. First, many of the productive PI's we identified became part of the germplasm collection in the 1930's or before, thus, they are not simply duplications of recently developed U. S cultivars, but in all likelihood are genetically distinct from current U. S. breeding material. Second, because northern U. S. soybean cultivars are derived almost exclusively from introductions originating in northeastern China, soybean plant introductions from other parts of the Far East will very likely offer unique and desirable allelles.

Poor visual seed quality resulting from infection by Diaporthe phaseolorum (Cke., and Ell.) Sacc. var. sojae (Lehman) Wehm and Cercospora kikuchii (Mat. and Tomoy) Chupp is a problem of particular importance in the Middle Atlantic soybean production area. The significantly better visual seed quality of the PI's as compared to the adapted genotypes in Experiment II and IV and the presence of the PI's in the four experiments with visual seed quality scores numerically superior to all entries suggests that genotypes with improved seed quality could be developed using selected PI's.

Although the limited genetic variation in adapted soybean breeding lines has apparently not curtailed long-term improvements in soybean yield potential, this possibility must be considered. The development of soybean populations using germplasm which is not currently included in breeding materials should be initiated so that population development can proceed and productive materials with acceptable lodging and shattering resistance, along with other agronomic characteristics, will be available in the future when genetic homogeneity may become a recognized hindrance to genetic progress. We suggest the development of a number of soybean populations using selected plant introductions from specific East Asian origins and also populations combining the most desirable PI's from Japan, Korea, or northeastern China. Careful selection of PI's for agronomic potential might obviate the necessity of including adapted genotypes in these populations. Selection in a population

containing a large proportion of adapted germplasm may tend to eliminate genetic diversity in favor of types which are phenotypically and genotypically similar to adapted parents. The exclusion of adapted genotypes also seems justified because a number of PI's yielded 90% or more of the adapted genotypes and thus populations of 100% PI germplasm could not possess drastically depressed yield potential. We are currently backcrossing the ms_2 gene (Bernard and Cremeens, 1975) into approximately 30 PI's to allow the synthesis of populations with PI germplasm in which intermating would be facilitated and the cytoplasm would be of PI origin.

References:

Bernard, R.L. and C.R. Cremeens. 1975. Inheritance of the Eldorado male sterile trait. Soybean Genet. Newsl. 2:37-39.

Boerma, H. R. 1979. Comparison of past and recently developed soybean cultivars in maturity groups VI, VII, and VIII. Crop Sci. 19:611-613.

Delannay, S., D.M. Rodgers, and R.G. Palmer. 1983. Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop Sci. 23:944-949.

Hartwig, E.E. 1973. Varietal development. In B.E. Caldwell (ed.) Soybeans: Improvement , Production, and Uses. Am . Soc. of Agron., Madison, WI.

Hymowitz, T., C.A. Newell, and S. Carmer. 1977. Pedigrees of soybean cultivars released in the United States and Canada. INTSOY Series #13, Urbana, IL.

Johnson, H.W., and R.L. Bernard. 1963., Soybean genetics and breeding. In A.G. Norman (ed.), The Soybean. Academic Press, New York, NY.

Kenworthy, W.J., and C.A. Brim. 1979. Recurrent selection in soybeans. I. Seed yield. Crop Sci. 19:315-318.

Luedders, V.D. 1977. Genetic improvement in yield of soybeans. Crop Sci. 17:971-972.

National Academy of Sciences. 1972. Genetic vulnerability of major crops. Washington, DC.

Schoener, C.S., and W.R. Fehr. 1979. Utilization of plant introductions in soybean breeding populations. Crop Sci. 19: 185-188.

Specht, J.E., and J.E. Williams. 1984. Genetic technology contributions to soybean productivity- retrospect and prospect .pp. 49-74. In W.R.Fehr (ed.), Genetic Contributions to Yield Gains of Major Crop Plants. ASA Spec. Publ. 7, Madison WI.

St. Martin, S.K. 1982. Effective population size for the soybean improvement program in maturity groups 00-IV. Crop Sci. 22:151-152.

Wilcox, J.R., W.T. Schapaugh, Jr., R.L. Bernard, R.L. Cooper, W.R. Fehr, and M.N. Niehaus. 1979. Genetic improvement of soybeans in the midwest. Crop Sci. 19: 803-805.

LIST OF TABLES

P.B. Cregan
J.O. Yocum
J.R. Justin
C.R. Buss
W.J. Kenworthy
E.L. Wisk
H.M. Camper Jr. 2/

1/ Paper no. D-15478-1-83 of the Journal Series of the New Jersey Agirc. Exp. Stn.

2/ Research geneticist, Nitrogen Fixation and Soybean Genetics Laboratory, USDA-ARS, Beltsville, MD 20705; senior research associate of Agronomy, The Pennsylvania State Univ., University Park, PA 16802; ext. specialist in Soils and Crops, Cook College, Rutgers Univ., New Brunswick, NY 08903; associate professor of Agronomy, Virginia Polytecnic Institute and State Univ., Blacksburg, VA 24061; associate professor of Agronomy, Univ. of Maryland, College Park, MD 20742; research associate, Univ. Substation, Delaware AES, Georgetown, DE 19947; and assistant professor of Agronomy , Eastern Virginia Research Station, Virginia AES, Warsaw, VA 22572, respectively.

Table 1. Adapted genotypes, ancestral cultivars and plant introductions grown in replicated performance trials, 1979 to 1981.

Experiment II	Experiment III		Experiment IV		Experiment V
Adapted genotypes					
Hawkeye	Adams	Elf	Calland	Union	Dyer
Harosoy	Wayne	A75-305022	Wabash	Kent	Hill
Amsoy 71	Lincoln		Clark	Perry	Essex
Beeson	Calland		Columbus		Forrest
Harcor	Williams		Cutler 71		Bay
Ancestral cultivars					
Mardarin (Ottawa)	AK (Harrow)		AK (Harrow)		
Manchu (Madison)	Dunfield		Dunfield		
Mukden	Mukden				
Richland					
Seneca					

Table 1 cont. Adapted genotypes, ancestral cultivars and plant introductions grown in replicated performance trials, 1979 to 1981.

Experiment II	Experiment III		Experiment IV			Experiment V
Plant introductions						
PI 54.620	PI 54.583(NC)+	PI 88.486 (NC)	FC 19.976-1	PI 157.449	PI 398.941	PI 84.949
PI 68.522	PI 54.592 (NC)	PI 89.143 (K)	FC 19.979-7	PI 219.782	PI 399.023	PI 96.983
PI 68.670-2	PI 68.398 (NC)	PI 92.645 (NC)	FC 31.685	PI 229.361	PI 399.027	PI 157.444
PI 68.683	PI 80.466-1 (J)	PI 92.686 (NC)	PI 54.610-4	PI 253.665B	PI 399.091	PI 181.558
PI 79.602	PI 80.471-1 (J)	PI 196.157 (J)	PI 61.944	PI 273.483D	PI 406.709	PI 229.327
PI 86.122	PI 84.611 (K)	PI 273.483B (K)	PI 61.947	PI 339.981	PI 407.845A	PI 248.511
PI 96.194-3	PI 84.656 (K)	PI 273.483F (K)	PI 76.696	PI 339.997	PI 407.932B	PI 324.924
PI 181.554	PI 84.987A (J)	PI 360.841 (O)	PI 80.834-1	PI 360.848	PI 408.092B	PI 339.978
PI 297.545	PI 85.356 (K)	PI 398.312 (K)	PI 81.034-1	PI 385.942	PI 408.100B	PI 339.999
PI 360.835	PI 86.063 (J)	PI 398.395 (K)	PI 82.218	PI 398.226	PI 408.110A	PI 398.433
PI 361.062B	PI 86.116 (J)	PI 398.494 (K)	PI 82.555	PI 398.318	PI 408.131A	PI 399.045
PI 361.065B	PI 86.144 (J)	PI 398.702 (K)	PI 85.469	PI 398.356	PI 408.196B	PI 407.913A
PI 361.116	PI 87.465-2 (K)	PI 398.930 (K)	PI 86.136	PI 398.379	PI 408.198	
PI 383.278	PI 87.634 (J)	PI 398.965 (K)	PI 86.740	PI 398.392	PI 408.225A	
PI 384.468	PI 88.287 (NC)		PI 87.059	PI 398.430	PI 408.269D	
	PI 88.305 (NC)		PI 87.620-1	PI 398.621	PI 408.270B	
	PI 88.306 (NC)		PI 88.302-1	PI 398.816	PI 408.318A	
	PI 88.310 (NC)		PI 157.404	PI 398.873	PI 408.335A	

+Denotes origin of plant introduction, NC= Northeastern China, J= Japan, K=Korea, O=Other

Table 2. Experimental designs, year, location, and mean yield performance of replicated plant introduction performance trials.

Maturity group and experiment	Entries No.	Experimental design	Year and trial location	Yield kg/ha
II	25	5 X 5 simple lattice	1979	
			Adelphia, NJ	1,875
			Landisville, PA	3,040
			Beltsville, MD	1,622
			1980	
			Adelphia, NJ	1,436
			Landisville, PA	2,050
			Clarksville, MD	2,101
			Beltsville, MD	2,448
III	42	6 X 7 rectangular lattice	1979	
			Adelphia, NJ	1,812
			Landisville, PA	2,376
			Beltsville, MD	1,514
			1980	
			Adelphia, NJ	1,219
			Landisville, PA	2,318
			Queenstown, MD	2,086
			1981	
			Beltsville, MD	2,367
			Queenstown, MD	2,563
IV	64	8 X 8 simple lattice	1979	
			Landisville, PA	2,530
			Beltsville, MD	1,987
			Queenstown, MD	2,139
			1980	
			Landisville, PA	2,273
			Queenstown, MD	2,000
V	17	Randomized complete block	1979	
			Beltsville, MD	1,610
			Queenstown, MD	2,167
			Warsaw, VA	2,464
			1980	
			Beltsville, MD	1,726
			Queenstown, MD	1,715
			Georgetown, DE	1,092

Table 3. Mean agronomic and seed quality characteristics of maturity group II soybean cultivars and plant introductions selected to represent the range of performance, 1979 and 1980.

Entries	Yield		Maturity	Lodging score	Visual seed quality score
Adapted genotypes	kg / ha	rank +	days after August 31	1-5	1-5
Amsoy 71	2,163 abc++	9	24.8 b-d	1.7 ij	3.4 a
Beeson	2,343 ab	2	22.4 e-f	1.6 j	2.8 c-g
Harcor	2,453 a	1	24.3 b-f	1.9 g-j	2.8 b-e
Harosoy	2,020 bc	15	20.8 g-j	1.5 j	2.9 a-d
Hawkeye	2,318 abc	4	23.1 d-g	1.9 g-j	2.5 c-g
Mean	2,260 a§		23.1 a	1.7 c	2.9 a
Ancestral cultivars					
Manchu	2,013 bc	16	20.4 g-j	2.5 c-f	2.8 c-f
Mukden	2,044 bc	14	23.1 d-g	1.9 g-j	2.5 c-g
Richland	1,959 bc	20	26.7 b	1.8 hij	3.4 ab
Seneca	1,926 cd	22	17.6 j	2.5 b-f	2.9 abc
Mean¶	1,986 b		22.0 a	2.2 b	2.9 a
Plant introductions					
PI 68.670-2	2,220 abc	8	24.4 b-f	2.7 b-e	2.6 c-g
PI 68.683	2,324 abc	3	22.5 e-h	2.8 bcd	2.7 c-g
PI 79.602	1,970 bc	19	21.4 f-i	3.4 a	2.9 a-d
PI 86.122	2,070 abc	13	29.7 a	2.7 b-e	2.5 c-g
PI 96.194-3	2,254 abc	6	26.4 bc	3.1 ab	2.2 fg
PI 181.554	2,090 abc	12	26.0 bcd	3.5 a	2.3 d-g
PI 360.835	2,140 abc	10	26.8 b	2.2 e-i	2.2 efg
PI 361.062B	2,250 abc	7	23.4 c-g	2.5 c-g	2.5 c-g
PI 361.065B	1,589 d	25	21.0 f-i	2.0 f-j	2.5 c-g
PI 361.116	2,119 abc	11	21.6 e-i	2.3 d-h	2.3 c-g
PI 383.278	1,953 bc	21	19.2 hij	3.0 abc	2.2 g
PI 384.468	2,291 abc	5	18.5 ij	1.6 j	2.4 c-g
Mean#	2,079 b		23.0 a	2.7 a	2.4 b

+ Based upon the 25 entries in Experiment II.

++ Individual entry menas within a column followed by the same letter are not significantly ($P \leq 0.05$) as tested by Duncan's Multiple Range Test.

§ Adapted genotype, ancestral cultivars and PI group means followed by the same letter are not significantly different as tested by "$t_{.05}$".

¶ Mean does not include, Mandarin (Ottawa).

Mean of 15 PI's included in Experiment II.

Table 4. Mean agronomic and seed quality characteristics of maturity group III adapted soybean genotypes. ancestral cultivars, and plant introductions selected to represent the range of performance.

Entries	Yield		Maturity	Lodging score	Visual seed quality score
Adapted genotypes	kg / ha	rank+	days after 8/31	1-5	1-5
A75-305022	2,459 ab++	2	30.5 e-i	1.9 i	2.4 c-h
Adams	2,176 a-f	8	22.1 n	2.4 ih	2.9 a-f
Calland	2,479 a	1	32.7 c-f	2.4 ih	2.7 a-g
Elf	2,432 abc	3	34.5 bcd	1.9 i	2.3 c-h
Lincoln	2,062 def	19	25.1 k-n	2.4 ih	2.7 a-f
Wayne	2,217 a-e	6	27.1 h-l;	2.4 ih	3.0 a-d
Williams	2,297 a-d	5	32.7 c-f	2.0 i	2.4 c-h
Mean	2,302 a§		29.3 b	2.2 c	2.6 a
Ancestral cultivars					
AK (Harrow)	2,171 a-f	9	23.6 lmn	3.1 d-g	2.7 a-f
Dunfield	1,877 fgh	34	23.7 imn	3.3 b-f	2.9 a-e
Mukden	2,140 c-f	12	22.9 mn	2.3 ih	2.2 f-h
Mean	2,062 b		23.4 c	2.9 b	2.6 a
Plant introductions					
PI 68.398	1,952 efg	27	33.9 b-e	3.2 d-g	2.9 a-f
PI 80.471-1	2,076 def	18	26.9 i-l	2.3 ih	2.7 a-f
PI 84.611	1,683 gh	40	30.1 f-i	4.0 a	2.9 a-e
PI 84.656	2,307 a-d	4	33.8 b-e	2.8 fgh	2.7 a-f
PI 84.987	1,992 def	24	28.6 g-k	3.1 efg	2.3 d-h
PI 85.356	2,143 c-f	11	30.0 e-i	3.8 abc	2.7 a-g
PI 86.063	1,967 efg	25	28.5 g-k	2.8 fgh	2.3 c-h
PI 86.116	1,962 efg	26	28.8 g-j	2.8 fgh	2.7 a-g
PI 88.287	1,642 h	42	27.9 h-k	2.9 fgh	3.3 ab
PI 88.305	1,645 h	41	39.5 a	3.3 c-g	2.9 a-e
PI 88.306	2,090 def	15	33.0 c-f	3.6 a-e	3.3 ab
PI 88.310	2,151 b-f	10	31.7 d-g	3.9 ab	3.0 abc
PI 88.486	2,207 a-e	7	30.7 e-h	2.7 gh	3.4 a
PI 92.686	2,059 def	21	25.6 j-m	2.8 fgh	2.6 b-h
PI 196.157	1,948 efg	28	29.8 f-i	2.5 ih	2.0 h
PI 398.494	2,087 def	16	34.7 bcd	3.7 a-d	2.0 gh
PI 398.702	1,920 e-h	29	36.7 ab	2.8 fgh	2.3 d-h
PI 398.965	1,883 fgh	33	35.6 bc	2.8 fgh	2.4 c-h
Mean¶	1,970 b		31.6 a	3.2 a	2.6 a

+ Based upon the 42 entries in Experiment III.

++ Individual entry means within a column followed by the same letter are not significantly different ($P \leq 0.05$) as tested by Duncan's Multiple Range Test.

§ Adapted genotype, ancestral cultivar, and PI group means followed by the same letter are not significantly different as tested by "$t_{.05}$".

¶ Mean of the 32 PI's in Experiment III.

Table 5. Mean performance of plant introductions from Japan, Korea, and northeastern China in Experiment III, 1979-1981.

PI origin	Genotypes	Yield	Maturity	Lodging score	Visual seed quality score
	No.	kg / ha	days after 8/31	1-5	1-5
Japan	8	1,940 b[+]	29.1 b	3.0 c	2.5 b
Korea	13	2,011 a	32.6 a	3.4 a	2.5 b
Northeastern China	10	1,932 b	31.9 a	3.2 b	2.9 a

[+] Means within a column not followed by the same letter are significantly different as tested by individual mean comparisons using "$t_{.05}$".

Table 6. Mean and range of agronomic and seed quality characteristics of adapted genotypes and plant introductions grown in Experiments IV and V, 1979 and 1980.

Experiment	Genotypes	Yield	Maturity	Lodging score	Visual seed quality score
	No.	kg / ha	days after 8/31	1-5	1-5
Experiment IV					
Adapted genotypes	10				
Mean		2,707 a	41.2 b	2.5 a	2.5 a
Range		2,448-2,903	38.4-46.9	2.0-2.9	2.2-2.9
Plant introductions	54				
Mean		2,106 b	44.4 a	3.5 b	2.4 b
Range		1,602-2,521	36.4-52.4	2.6-4.5	1.9-2.9
Experiment V					
Adapted genotypes	5				
Mean		1,975 a	54.1 a	3.0 a	2.1 a
Range		1,763-2,163	51.1-57.9	2.5-3.5	2.0-2.3
Plant introductions	12				
Mean		1,721 b	52.2 b	3.0 a	2.1 a
Range		1,456-1,909	48.2-56.5	2.6-3.4	1.9-2.6

[+] Means within a maturity group within a column followed by the same letter are not significantly different as tested by "$t_{.05}$".

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Plant Molecular Biology Laboratory
Plant Sciences Institute
Beltsville, Maryland 20705

Tests for genetic linkage of the *Fr2*, *Pc*, and *I* loci in soybean

The development of a classical genetic map of soybean is still at a rudimentary stage. Although 20 linkage groups have been defined in soybeans, most of these linkage groups consist of only two linked genes. Numerous genes have yet to be mapped and located in particular linkage groups. In order to distinguish separate chromosome blocks, it is equally important to establish which loci segregate independently. In this study, we tested the *Fr2* gene (controlling fluorescence of the soybean root in UV light) and the *Pc* gene (controlling curly pubescence) and the *I* gene (controlling the inhibition of pigment in the seed coat) for genetic linkage or independent assortment. The cross of T141 (*Fr2, pc, i-i)* x PI290136 (*fr2, Pc, i*) was made in the field at Beltsville, Maryland. Both parental lines were obtained from the USDA Soybean Germplasm Collection at Urbana, Illinois. The gene symbols refer to the genetic traits described by Palmer and Kilen (1987).

The F_2 seed were produced at Beltsville, Maryland. F_2 seeds were germinated in petri dishes and the seedlings were classified as fluorescent or non fluorescent by response to UV illumination 1-2 days after initiation of imbibition. After classification, seedlings were transplanted to clay pots filled with greenhouse potting soil. After five weeks, they were tagged and the pubescence characteristic was recorded on the tag. At maturity, plants were classified for self-colored vs. non self-colored seed. Recombination estimates were calculated from the F_2 data using the method of maximum likelihood as described by Allard (1956) and Mather (1951). The bisection method (Yakowitz and Szidarovsky, 1989) was used to solve the maximum likelihood equations.

Chi-square for monogenic ratios obtained for all the gene loci indicated a good fit to a 3:1 ratio. The Chi-square for linkage was partitioned from the total Chi-square by subtraction of the Chi-square for each of the monogenic ratios composing the total Chi-square. The Chi-square for linkage indicated independent assortment of *Fr2* vs. *Pc*, *Fr2* vs. *I* and *Pc* vs. *I* (Table 1).

The *I* locus has previously been located in classical linkage group VII (Weiss, 1970), 17.8 map units from the *O* locus and 41.1 map units from the Y13 locus. The *I*

locus is also known to be very tightly linked to the *Rhg4* locus, controlling resistance to the soybean cyst nematode (Matson and Williams, 1965). Two RFLP molecular markers have been located close to and straddling the *I* locus (Weismann et al., 1992). the *I* locus is also known to be located in linkage group A of the molecular map being developed by the USDA at Iowa State (Keim et al., 1989). The *Fr2* and *Pc* loci are most unlikely to be linked to the loci identified as closely linked to the *I* locus. The loci linked to the *I* locus, but not closely linked, are less likely to be linked to the *Fr2* and *Pc* loci.

Acknowledgments: The assistance of Sean M. Fox, a volunteer from Calvert High School, is gratefully acknowledged.

References:

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235-278.

Keim, P., B.W. Diers, R.G. Palmer, R.C. Shoemaker, T. Macalma, K.G. Lark. 1989. Mapping the soybean genome with RFLP markers. pp. 1246-1251. In: A. J. Pascale (ed.) Proc. 4th World Soybean Res. Conf. Buenos Aires, Argentina.

Mather, K. 1951. The measurement of linkage in heredity. 2nd ed. Methuen & Co., London.

Matson, A. L. and L.F. Williams. 1965. Evidence of the fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:477.

Palmer, R.G. and T.C. Kilen. 1987. Qualitative genetics and cytogenetics. p. 135-209. In J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16:ASA, CSSA and SSSA, Madison, WI.

Weisemann, J.M., B.F. Matthews and T.E. Devine. 1992. Molecular markers located proximal to the soybean cyst nematode resistance gene, Rhg4. Theor. App. Genet. 85:136-138.

Weiss, M.G. 1970. Genetic linkage in soybeans: Linkage group VII. Crop Sci. 10:627-629.

Yakowitz, S. and F. Szidarovszky. 1989. An introduction to numerical computations. 2nd ed. Macmillan Publishing, New York.

T.E. Devine

Table 1. Soybean genetic linkage tests with the cross T141 (pc, Fr_2, i-i) x PI290136 (Pc, fr_2, i)

	Genotype †									
Genes	a	b	c	d	Sum	X2L*	P(X2L)§	Rec¶	SE#	Phase††
FR_2, Pc	105	25	34	7	171	.053	.07-.09	48	6	R
Fr_2, I	99	30	33	8	170	.212	.05-.07	53	6	C
Pc, I	103	30	26	5	164	.271	.05-.07	46	6	R

† Class designations per Allard, 1956.
* Linkage Chi-square tests were calculated using the method of maximum likelihood with a 9:3:3:1 ratio with 1 df.
§ Chi-square probability
¶ Rec = estimate of the recombination frequency using the method of maximum likelihood
# SE = standard error or recombination estimate
†† Phase: C = Coupling, R = Repulsion

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN
Department of Agronomy

Inheritance of Brown Stem Rot Resistance in PI 437685D

Introduction: Brown stem rot caused by Phialophora gregata (Allington and Chamberlain) W. Gams, has been reported throughout the midwestern and southeastern United States. P. gregata is a soil borne pathogen that enters soybean through main and lateral roots (Allington and Chamberlain, 1944). During environmental conditions favorable to P. gregata growth, conidia and mycelium spread throughout the plant causing browning of the vascular system. Infected plants will develop interveinal leaf necrosis followed by a wilting and drying of leaves (Abel, 1977). Infection of P. gregata can result in soybean yield reduction of 12 to 38% (Gray, 1972; and Sebastian et al., 1986).

Currently, three dominant genes conferring resistance to P. gregata have been reported. The Rbs1 gene was identified in the breeding line L78-4094 (Hanson et. al., 1988; Sebastian and Nickell, 1985), which derives its resistance to brown stem from PI 84946-2. Sebastian and Nickell (1985) suggested that PI 84946-2 contains the Rbs1 and possibly a second gene for brown stem rot resistance. The Rbs2 gene (Hanson et al., 1988) and Rbs3 gene (Willmot and Nickell, 1989) were identified in PI 437833 and PI 437970, respectively. Recently, Waller et al. (1991) suggested that polygenic resistance occurs in Asgrow A3733, which does not derive its resistance to brown stem rot from known sources of resistance. At the present time, all publicly released cultivars with brown stem rot resistance derive their resistance from PI 84946-2. Since variability in pathogenicity of P. gregata isolates has been reported (Willmot, 1988), additional sources of resistance would be beneficial to breeding programs.

In 1989, Nelson et al. identified that PI 437685D was resistant to P. gregata at Hancock, Wisconsin. Willmot et al., (1989) determined that PI 437685D contained a single dominant gene that was not allelic to the Rbs1 and Rbs2 genes. Therefore, the objective of this research was to study the allelic relationship of the Rbs3 gene to the single dominant gene in PI 437685D.

Materials and Methods: During the winter of 1991-1992, F_2 and $F_{2:3}$ plants from the cross of PI 437685D x PI 437970 were evaluated in the greenhouse for brown stem rot resistance. Sixty F_2 plants and 40 $F_{2:3}$ families of 35 plants per family were evaluated using a root dip screening procedure similar to that used by Willmot and

Nickell (1989).

Approximately six weeks after inoculation, plants were evaluated for the percentage of total nodes showing leaf symptoms and internal stem browning. Plants were classified as susceptible if their brown stem rot symptoms were greater than the lower 95% confidence limit of brown stem rot symptoms on the cultivar Century 84 (Walker et al., 1986). 'Century 84' is susceptible to P. gregata and was used as the susceptible standard.

Observed segregation ratios were tested against theoretical inheritance ratios using chi-square analysis to determine the best inheritance model. A duplicate dominant epistatic model would be expected if the single dominant gene in PI 437685D was not allelic to the Rbs3 gene.

Results and Discussion: In the F_2 generation, no plants susceptible to P. gregata were observed. In the F_3 generation, approximately 3.0% of the plants were classified as susceptible, but individual F_3 plants did not fit the expected 55:9 (resistant:susceptible) ratio. In the $F_{2:3}$ families, no susceptible families or families segregating 3:1 (resistant:susceptible) were observed (Table 1). Therefore, it was concluded that F_3 plants classified as susceptible were resistant genotypes that had a large environmental variance. From the histograms of F_3 plants (Fig. 1), it is obvious that no distinct group of susceptible genotypes exists, but only a gradual decrease in the number of plants classified as susceptible. Thus, it is concluded that PI 437685D contains a single dominant gene not allelic to the Rbs1 or Rbs2 genes (Willmot et al., 1989), but allelic to the Rbs3 gene. Therefore, PI 437685D does not represent a new source of single gene resistance to brown stem rot when compared to PI 437970. However, PI 437685D provides the Rbs3 gene in an additional unique genotype that may be more advantageous in certain breeding situations.

References

Abel, G. M. 1977. Brown stem rot of soybean-Cephalosporium gregatum. Rev. Pl. Path. 56:1065-1077.

Allington, W. B., and D. W. Chamberlain. 1948. Brown stem rot of soybean. Phytopath. 38:793-802.

Gray, L. E. 1972. Effect of Cephalosporium gregatum on soybean yield. Pl. Dis. Rep. 56:580-581.

Hanson, P. M., C. D. Nickell, L. E. Gray, and S.A. Sebastian. 1988. Identification of two dominant genes conditioning brown stem rot resistance in soybean. Crop Sci. 28:41-43.

Nelson, R. L., C. D. Nickell, J. H. Orf, H. Tachibana, E. T. Gritton, C. R. Grau, and B. W. Kennedy. 1989. Evaluating soybean germplasm for brown stem rot resistance. Pl. Dis. 73:110-114.

Sebastian, S. A., and C. D. Nickell. 1985. Inheritance of brown stem rot resistance in soybeans. J. Hered. 76:194-198.
Sebastian, S.A., C. D. Nickell, and L. E. Gray. 1986. Relationship between greenhouse and field ratings for brown stem rot reaction in soybean. Crop Sci. 26:665-667.
Waller, R. S., C. D. Nickell, D. L. Drzycimski, and J. E. Miller. 1991. Genetic analysis of the inheritance of brown stem rot resistance in the soybean cultivar Asgrow A3733. J. Hered. 82:412-417.
Walker, A. K., A. F. Schmitthenner, R. J. Fioritto, S. K. St. Martin, R. L. Cooper, and R. J. Martin. 1986. Registration of 'Century 84' soybean. Crop Sci. 26:199-200.
Willmot, D. B., and C. D. Nickell. 1989. Genetic analysis of brown stem rot resistance in soybean. Crop Sci. 29:672-674.
Willmot, D. B., C. D. Nickell, and L. E. Gray. 1989. Physiologic specialization of Phialophora gregata on soybean. Pl. Dis. 73:290-294.
Willmot, D. B., D. H. Horsch, R. S. Waller, and C. D. Nickell. 1989. Preliminary study on the inheritance of brown stem rot resistance PI 437685D. Soybean Genet. Newsl. 16:99-102.

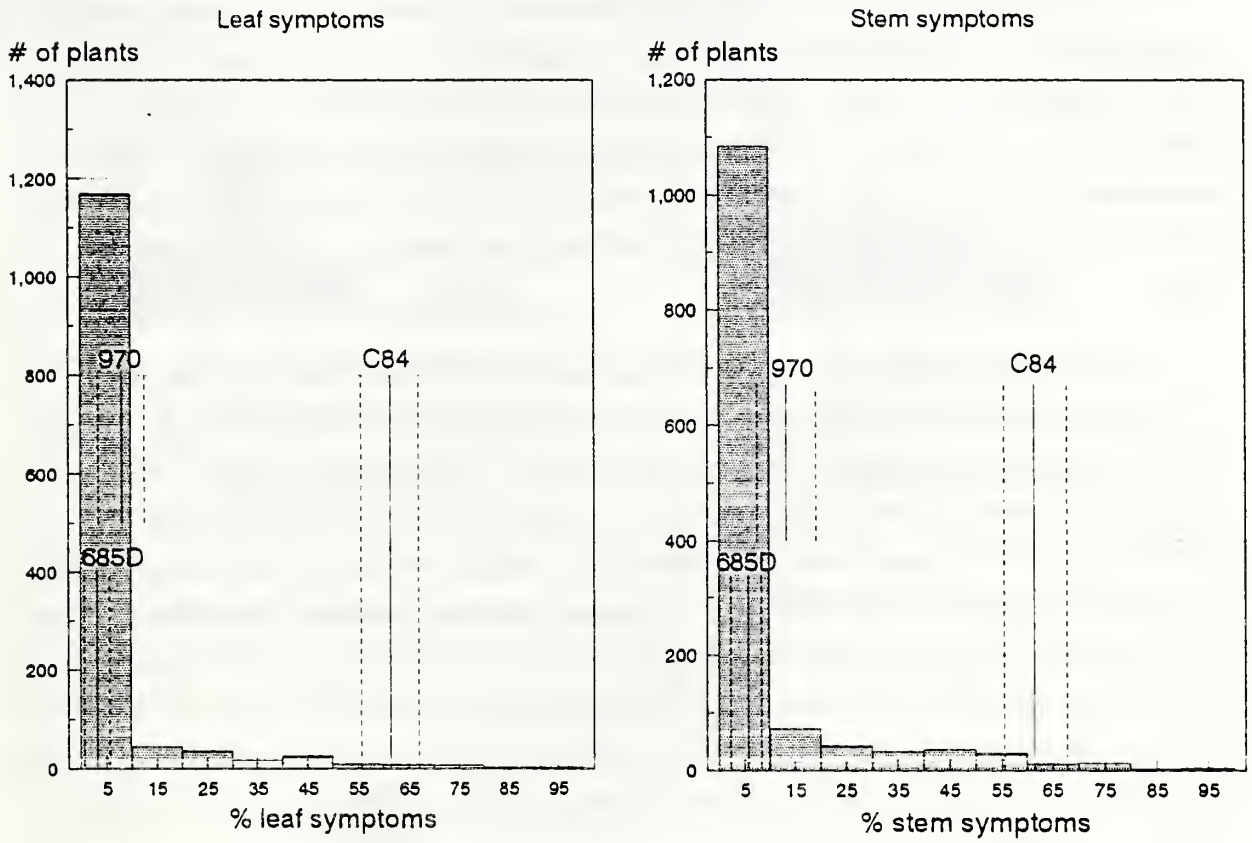
S. R. Eathington
C. D. Nickell

Table 1. Results of F_2 and F_3 generations of the cross between PI 437685D x PI 437970. Plants were classified as resistant or susceptible by the lower 95% confidence limit of symptoms on Century 84.

Symptoms	Gen.†	Checks	Checks mean‡	Checks std. err.§	Res.¶	Seg.#	Sus.††	Ratio tested	Chi-square	p value
			— % —		———	no.	———			
Leaf	F_2				59		0	15:1	2.94	0.09
		Century 84	72.6	6.3						
		PI 437970	1.8	1.2						
		PI 437685D	0.0	0.0						
	$F_{2:3}$ families				40	0	0	11:4:1	18.18	0.00
	F_3				1300		32	55:9	149.85	0.00
		Century 84	61.2	2.8						
		PI 437970	8.1	2.3						
		PI 437685D	3.0	1.1						
Stem	F_2				59		0	15:1	2.94	0.09
		Century 84	86.1	5.5						
		PI 437970	7.1	2.8						
		PI 437685D	4.4	4.4						
	$F_{2:3}$ families				40	0	0	11:4:1	18.18	0.00
	F_3				1284		48	55:9	103.57	0.00
		Century 84	61.5	3.0						
		PI 437970	13.6	2.8						
		PI 437685D	5.3	1.7						

† Generation
‡ Mean of symptoms observed.
§ Standard error of symptoms observed.
¶ Resistant genotypes and families that are resistant or segregating 15:1 (resistant:susceptible).
# Families segregating 3:1 (resistant:susceptible).
†† Susceptible genotypes and families that are susceptible.

Fig. 1. Histograms of F_3 plants for leaf and stem symptoms from the cross of PI 437685D x PI 437970. Solid lines are the mean value and the dotted lines are the 95% confidence interval. The symbols C84, 970, and 685D represent Century 84, PI 437970, and PI 437685D, respectively.



S. R. Eathington
C. D. Nickell

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
P.O. Box 1071, Knoxville, TN 37901-1071

Progress report on the construction of a yeast artificial chromosome library of soybean

We have begun the construction of libraries of soybean DNA in yeast artificial chromosomes. Two libraries are being built in parallel. One uses the vector YAC4 and contains fragments of soybean DNA generated by partial digestion with EcoRI. The other is being constucted in YAC55. Using size selected SalI digested DNA, we are attempting to clone a specific fragment containing the marker pUTG-132a, which has been mapped to within 1cM of the nts locus (Landau-Ellis et al., 1991; Carroll et al., 1985).

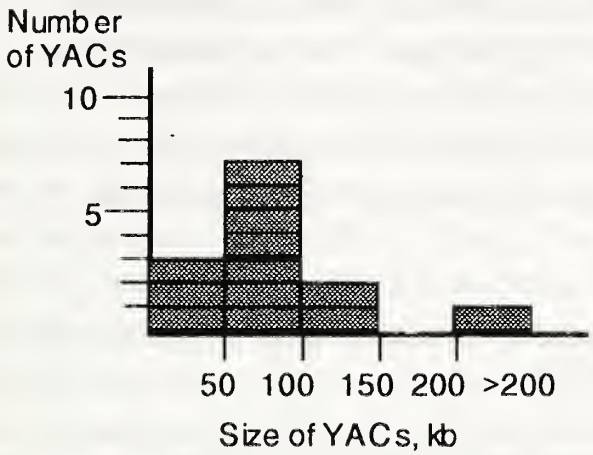
Our starting material is high molecular weight soybean DNA prepared from protoplasts embedded in agarose blocks (Honeycutt et al., 1992; Funke et al., 1993). The DNA is partially digested with EcoRI and ligated to dephosphorylated YAC vector directly, or following a size selection step on a CHEF gel. In common with the experience of other researchers (eg Kleine et al., 1993), we found that the introduction of size selection steps in an attempt to increase the average insert size of the artificial chromosomes reduces the overall efficiency of the procedure (Fig. 1). Without size selection we obtained numerous clones but the average insert size was only 90 kb. Selection of partial digestion products on a CHEF gel before ligation and transformation increased the average insert size of the clones to 160 kb, at the expense of a 10-fold drop in transformation efficiency.

We have stored 600 size selected YACs and are experimenting with modifications in the procedure to reach a satisfactory compromise between insert size and transformation efficiency. 5% of clones hybridized to a chloroplast-specific probe.

Most of the YACs hybridize with varying degrees of intensity to labelled total soybean DNA, due to their repetitive DNA contents. Because there is evidence that repetitive DNA sequences can affect the stability of YAC clones during propagation in yeast , we checked the stability of four of the clones representing a range of repetitive DNA contents. We mimicked the process of storing and duplicating the clones several times and grew them up in rich and selective media. The size of all the clones remained

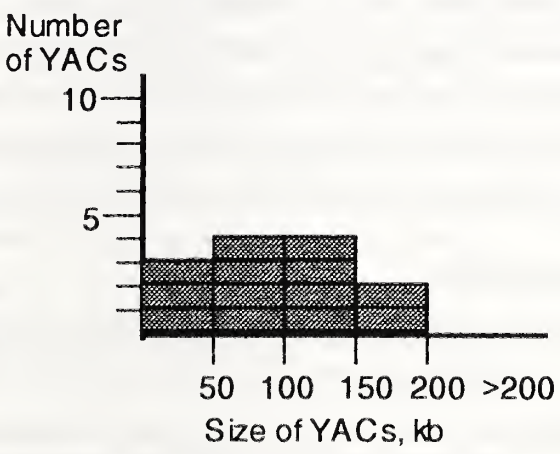
stable, showing that even clones with a high fraction of repetitive DNA are not especially susceptible to rearrangements or loss of sequences.

Unselected, with polyamines



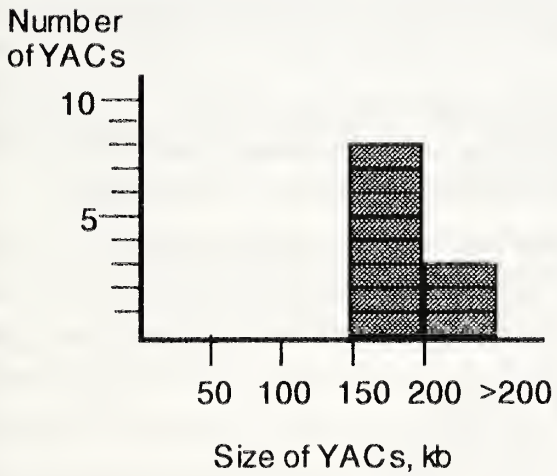
Average size 85 K

Unselected, no polyamines



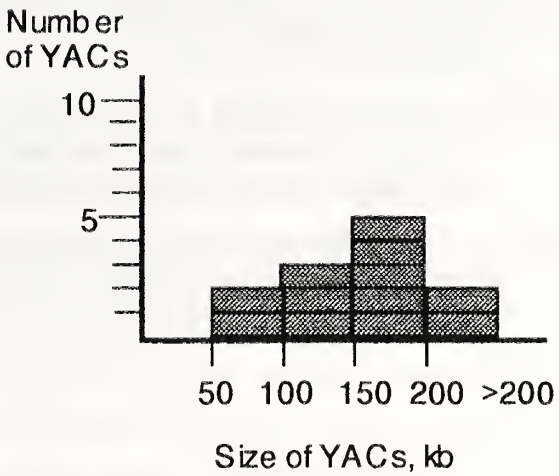
Average size 100 K

Selected, with polyamines



Average size 185 K

Selected, no polyamines



Average size 140 K

Chromosome walking strategies might be foiled in a large plant genome because of repetitive sequences. Repetitive sequences at the ends of clones would make it difficult to identify overlapping or contiguous clones. We isolated four ends of soybean YACs using the vectorette procedure (Riley et al., 1990). When these were hybridized back to genomic soybean DNA, one proved to be highly reiterated in the genome, one was intermediate, and two were unique. None of the end clones showed polymorphisms between Glycine max and G. soja in EcoRI and HindIII digests. Thus while it would be preferable to "land" on particular YACs containing markers of interest, and it is worthwhile to invest extra effort in the improving insert size, about half the end fragments of the YACs are useful for the purpose of identifying overlapping clones.

References:

Carroll, BJ, McNeil, DL, Gresshoff, PM: A supernodulation and nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78: 34-40 (1985)

Funke, RP, Kolchinsky, A, Gresshoff, PM: Physical mapping in a region of the soybean genome containing duplicated sequences. Plant Mol. Biol. 22:437-446 (1993)

Honeycutt, RJ, Sobral, BWS, McClelland, M, Atherly, AG: Analysis of large DNA from soybean (Glycine max L. Merr.) by pulsed-field gel electrophoresis. The Plant Journal 2: 133-135.

Kleine, M, Michalek, W, Graner, A, Herrmann, R, Jung, C: Construction of a barley (Hordeum vulgare L.) YAC library and isolation of a Hor1-specific clone. Mol Gen Genet 240: 265-272 (1993).

Landau-Ellis, D, Angermüller, S, Shoemaker, R, Gresshoff, PM: The genetic locus controlling supernodulation in soybean (Glycine max L.) co-segregates tightly with a cloned molecular marker. Mol Gen Genet 228: 221-226 (1991).

Riley, J. Butler, R, Ogilvie, D, Finniear, R, Jenner, D, Powell, S, Anand, R, Smith, JC, Markham, AF: A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acids Res: 2887-2890 (1990).

R.P. Funke
A. Kolchinsky
P. M. Gresshoff

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Blacksburg, Virginia 24061
and
Plainview, Texas 79072

An abundant mitochondrial DNA species from *Glycine latifolia*.

Introduction: Restriction endonuclease digestion of plant mitochondrial DNA yields complex patterns of DNA fragments due to large genome sizes and the presence of repeated sequences. Non-stoichiometric fragments include both over-represented bands and those termed "sublimons" that are under-represented with respect to the majority of restriction fragments (Small *et al.* 1987). Plasmid-like molecules have also been observed in mitochondrial genomes of maize and other plants, including both circular and linear species (Kemble and Bedbrook 1980; Palmer *et al.* 1983; Pring *et al.* 1977). Much interest has centered on the maize linear plasmids S1 and S2 of the "S"-type cytoplasm for their association with the phenotype of cytoplasmic male sterility (Escote *et al.* 1985, Levings *et al.* 1980; Schardl *et al.* 1984). The plasmids exist both as free linear molecules or integrated within the complex mitochondrial genome. An 11.3 kb linear mitochondrial plasmid has been described in the genus *Brassica* that shows no homology to the large mitochondrial genome (Palmer *et al.* 1983). In a survey of soybean mitochondrial DNAs conducted to examine cytoplasmic diversity, undigested DNA samples examined under UV light in ethidium bromide-stained agarose gels showed a brightly fluorescing band in mtDNA from P.I. 253.238. From preliminary restriction mapping and Southern analysis we speculate that this DNA is a linear molecule. This is the first observation of an abundant, plasmid-like mitochondrial DNA species in soybean.

Materials and Methods: Mitochondrial DNA (mtDNA) preparations were isolated from etiolated soybean hypocotyls as previously described (Grabau *et al.* 1989). Undigested and restriction endonuclease-digested mtDNAs were subjected to electrophoresis in 0.7% agarose gels. After separation of DNA fragments, gels were stained with ethidium bromide and photographed prior to transfer to nylon filters by capillary blotting. Filters were subjected to Southern hybridization as previously described (Grabau *et al.* 1989). The undigested plasmid-like DNA was gel-purified for use as the hybridization probe. Filters were exposed to Kodak XAR5 film to produce an autoradiogram which was scanned with a Molecular Dynamics densitometer to give the results shown in Figure 1.

Results and Discussion: In a survey of soybean plant introductions of *Glycine soja* (Grabau and Davis 1992) and several wild perennial relatives, we observed one mtDNA sample that contained an abundant DNA species that migrated ahead of the main mtDNA band in a gel of undigested samples. Analysis of mtDNA in agarose gels was used as a first step in the

diversity studies to determine the quantity and quality of DNA preparations prior to endonuclease digestion. P.I. 253.238, which is classified as *Glycine latifolia* (T. Hymowitz, personal communication), contained a plasmid-like DNA species with an apparent mobility of approximately 13-14 kb. The fact that the molecule migrated as a single species led us to believe that this DNA existed in a linear conformation. Under the isolation procedures used for the mtDNA samples, it would be very unlikely for all the large circular molecules to have survived completely intact. If the DNA species were circular, we would have expected bands at both a circular and "broken circle" (linear) position. To further address the size and conformation of the molecule, we carried out a series of restriction endonuclease digestions and observed fragments that fluoresced brightly over the background of the normal mtDNA pattern of ethidium-bromide stained mtDNA. The sum of these fragments in each digestion agreed with the size prediction from the undigested abundant DNA species. We further concluded that the plasmid-like molecule was a unique linear species rather than a collection of broken circles because the restriction fragment products were discrete bands rather than a smear of fragment sizes.

To determine whether the sequence contained within the abundant DNA was unique or repeated within the main mitochondrial or nuclear genomes and to give an accurate size determination, we excised the undigested band from an agarose gel for use as a probe of restriction endonuclease-digested mtDNA. The densitometer scan of the results from the Southern analysis showed that there was homology to the probe only at positions already defined by the brightly fluorescing bands in the gel (Figure 1). We conclude that there is little or no sequence homology between the main mitochondrial genome and the large, plasmid-like molecule. The average size calculated from the total of the fragments was 13.4 kb. The fact that the bands on the autoradiogram were also of discrete sizes agreed with the conclusion that the single 13.4 kb undigested plasmid band was not a population of broken circular molecules.

The mitochondrial isolation procedure employed for these soybean samples gives a rather crude mtDNA preparation and some contaminating genomic DNA is routinely observed (Grabau *et al.* 1989). The fact that the use of the 13.4 kb molecule as a probe did not show hybridization to bands other than the abundant plasmid fragments also indicates that there are no repeated regions of homology to the plasmid-like molecule in the nuclear genome. It does not eliminate the possibility of only a single or very few copies of the plasmid-like sequence within the soybean nuclear genome.

We have examined over 600 mtDNA samples from the genus *Glycine* in order to classify them by cytoplasmic type and to search for additional sources of cytoplasmic diversity. P.I. 253.238 is the only sample from our survey that contains an large, abundant, plasmid-like mtDNA species.

Figure 1. Densitometer scan of an autoradiogram from P.I. 253.238 mtDNA. Total mtDNA was digested with restriction endonucleases and subjected to Southern analysis. A gel-purified 13.4 kb plasmid-like DNA species was used as the hybridization probe of the following single or double digests: 1) *Hin*dIII, 2) *Eco*RI, 3) *Hin*dIII/*Eco*RI, 4) *Bam*HI, 5) *Bam*HI/*Hin*dIII, 6) *Bam*HI/*Eco*RI, 7) *Sal*I, 8) *Xho*I. The position of the band in the *Xho*I digest was identical to the position in the undigested sample, indicating there is no *Xho*I site within the molecule. The autoradiogram was intentionally over-exposed to allow detection of the smallest restriction fragments but also reveals partial digestion in some of the single digestion lanes, *Bam*HI and *Sal*I in particular.

References:

Escote, L.J., Gabay-Laughnan, S.J. and Laughnan, J.R. (1985). Cytoplasmic reversion to fertility in *cms-S* maize need not involve loss of linear mitochondrial plasmids. Plasmid *14*: 264-267.

Grabau, E.A., and Davis, W.H. (1992). Cytoplasmic diversity in *Glycine soja*. Soybean Genetics Newsletter *19*: 140-144.

Grabau, E.A., Davis, W.H. and Gengenbach, B.G. (1989). Restriction fragment length polymorphism in a subclass of the `Mandarin' soybean cytoplasm. Crop Sci. *29*: 1554-1559.

Kemble, R.J. and Bedbrook, J.R. (1980). Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm. Nature *284*: 565-566.

Levings, C.S. III, Kim, B.D., Pring, D.R., Conde, M.F., Mans, R.J., Laughnan, J.R. and Gabay-Laughnan, S.J. (1980). Cytoplasmic reversion of *cms-S* in maize: association with a transpositional event. Science *209*: 1021-1023.

Palmer, J.D., Shields, C.R., Cohen, D.B. and Orton, T.J. (1983). An unusual mitochondrial DNA plasmid in the genus *Brassica*. Nature *301*:725-728.

Pring, D.R., Levings, C.S. III, Hu, W.W.L. and Timothy, D.H. (1977). Unique DNA associated with mitochondria in the "S"-type cytoplasm of male-sterile maize. Proc. Nat. Acad. Sci. USA *74*: 2904-2908.

Schardl, C.L., Lonsdale, D.M., Pring, D.R. and Rose, K.R. (1984). Linearization of maize mitochondrial chromosomes by recombination with linear episomes. Nature *310*: 292-296.

Small, I.D., Isaac, P.G. and Leaver, C.J. (1987). Stoichiometric differences in DNA molecules containing the *atpA* gene suggest mechanisms for the generation of mitochondrial genome diversity in maize. EMBO J. *6*: 865-869.

Elizabeth A. Grabau
William H. Davis

UNIVERSITY OF CENTRAL OKLAHOMA
Department of Biology
Edmond, Oklahoma 73034

Hypothetical mechanism for chlorophyll abnormalities in mutant soybean [*Glycine max* (L.) Merr.] lines

Altered isozyme banding patterns for NAD-malate dehydrogenase (E.C. 1.1.1.37; abbreviation = MDH) have been observed in soybean [*Glycine max* (L.) Merr.] lines demonstrating chlorophyll abnormalities (Palmer et al., 1989; Hedges, 1989; Amberger, 1990). Mutant soybean cultivars including chlorophyll-deficient (CD)-1, CD-2, and CD-3 (from Asgrow mutable; w4-m) (Palmer et al., 1989; Hedges, 1989) and mutant Jilin 3 (from Jilin 3) (Amberger, 1990), can be distinguished from wild-type (normal) cultivars through exhibition of yellow-green to yellow leaf pigmentation as well as zymograms lacking two of three mitochondrial bands ($mMDH^{(-)}$). Whether or not the chlorophyll abnormalities in these lines can be attributed to lack of mMDH isozyme bands is unknown.

Physiological and genetic mechanisms relating MDH deficiency to chlorophyll abnormality have been postulated (Bidlack et al., 1991). The physiological mechanism is based on a decrease in MDH activity and transport of malate and glutamate (via TCA cycle and glyoxylate pathway) to restrict chlorophyll synthesis; the genetic mechanism hypothesizes a transposable element that may alter genetic structure of associated genes for mMDH and chlorophyll synthesis. While both mechanisms provide an explanation of chlorophyll abnormalities, neither has been supported with concrete physiological or genetic data. The purpose of this paper is to provide a scheme that can be proven or disproved as the physiological basis for mMDH-imposed chlorophyll deficiency in mutant soybean lines.

Because MDH catalyzes one of the regulatory sites in the TCA cycle (Goodwin and Mercer, 1983; Gietl, 1992), it is possible that the lack of mMDH isozymes restricts

production of other TCA intermediates. Among these intermediates is a-ketoglutaric acid, an important precursor of glutamic acid, which is one of the essential intermediates in chlorophyll biosynthesis (Beale, 1990). Interrelationships among metabolites in the mitochondrion, cytosol, and chloroplast are shown by dark arrows in Figure 1. If the lack of two mMDH bands has the ability to slow down production of a-ketoglutarate, then it is possible that subsequent production of glutamate, and eventually chlorophyll, could be restricted. Thus, the lack of green pigmentation in the mutant plants may be a result of physiologically-restricted chlorophyll biosynthesis.

Another pathway indicating the relationship between MDH and chlorophyll biosynthesis is shown by light arrows in Figure 1. Organelle-bound MDH isozymes contained within the glyoxisome (gMDH) (Gietl and Hock, 1982) may restrict the formation of glyoxylate intermediates. Chlorophyll abnormalities may be attributed to lack of MDH in the glyoxisome and the chloroplast (cMDH), as well as in the mitochondrion. In both the TCA and glyoxylate cycles, conversion of malate to oxaloacetate can subsequently take one of two paths. The first allows oxaloacetate to be retained within the cycles themselves and be converted into citrate. The second path converts oxaloacetate into aspartate, which, in turn, is cycled back to malate in the TCA or glyoxylate cycles (Gietl, 1992). This pathway is coupled with the conversion of a-ketoglutarate to glutamate through transamination (Tolbert, 1979). The mechanism by which the MDH affects chlorophyll is mediated by transfer of malate, aspartate, glutamate, and a-ketoglutarate, via the malate-aspartate shuttle that resides in the chloroplast membrane. Shuttles of this type may also be mediated by peroxisomal intermediates, as indicated in Figure 1. The malate-aspartate shuttle is coupled in a clock-like fashion to the Calvin cycle (Tolbert, 1979; Goodwin and Mercer, 1983) and the contribution of glutamate (from the malate-aspartate shuttle) to synthesis of tetrapyroles. The clock-like mechanism that ties the malate-aspartate shuttle to the Calvin cycle is NADH and NADPH linked. Figure 1 shows an interactive, cyclic mechanism with the Calvin cycle and the malate-aspartate shuttle working together for the production of NADH and NADPH. If this flow of

metabolites is restricted by lack of mitochondrial, glyoxisomal, or even peroxisomal MDH, then the Calvin cycle and the donation of glutamate to tetrapyrrole synthesis are also restricted. Restriction of tetrapyrrole synthesis, indirectly via the malate-aspartate shuttle, would account for chlorophyll abnormalities in mutant soybean lines.

Restrictions of other pathways in soybean lines demonstrating chlorophyll abnormalities surely exist. Those outlined above are merely suggestions that stand to be proven or disproved by zymogram and enzyme data collected in the future. There are other enzymes involved in chlorophyll production that may play important roles in mutant soybean lines demonstrating chlorophyll abnormalities. These enzymes include glutamate 1-phosphotransferase (GPT), glutamate 1-semialdehyde:$NADP^+$ oxidoreductase (GSO), and glutamate 1-semialdehyde aminotransferase (GSA), and are involved in the conversion of glutamic acid into ∂-aminolaevulinic acid (Goodwin and Mercer, 1983; Beale, 1990). Isozyme analyses of GPT, GSO, and GSA may reveal altered zymograms in the mutant soybean lines.

References:

Amberger, L.A. 1990. Analyses of somaclonal variation in soybean [Glycine max (L.) Merr.]. Master's thesis. Iowa State Univ., Ames, IA.

Beale, S.I., 1990. Biosynthesis of the tetrapyrrole pigment precursor, aminolevulinic acid, from glutamate. Plant Physiol. 93:1273-79.

Bidlack, J.E., L.A. Bidlack, W. Caire, R.G. Palmer, and R.C. Shoemaker. 1991. Subcellular enzyme activities of mutant soybean lines lacking mitochondrial MDH isozyme bands. Soybean Genet. Newsl. 18:319-323.

Evans H.G., and N.G. Carr. 1979. The interaction of respiration and photosynthesis in microalgae. Encyclo. Plant Physiol. 6:163-173.

Gietl, C., 1992. Malate dehydrogenase isozymes: Cellular locations and ros are le in the flow of metabolites between the cytoplasm and cell organelles. Biochim. Biophys. Acta 1100:217-234.

Gietl C. and B. Hock. 1982. Organelle-bound malate dehydrogenase isozymesynthesized as higher molecular weight precursors. Plant Physiol. 70:483-487.

Goodwin T.W. and E.I. Mercer. 1983. Introduction to plant biochemistry. Pergamon Press. New York.

Hedges, B.R. 1989. The application of primary trisomics and transposon-induced mutations in genetic studies of soybean [Glycine max (L.) Merr.]. Ph.D. dissertation. Iowa State Univ., Ames, IA.

Palmer, R.G., B.R. Hedges, R.S. Benavente, and R.W. Groose. 1989. *w4*-Mutable line in soybean. Develop. Genet. 10:542-551.

Tolbert N.E. 1979. glycolate metabolism by higher plants. Encyclo. Plant Physiol. 6:339-352.

Aaron M. Elmer
Carrie A. Barton
James E. Bidlack

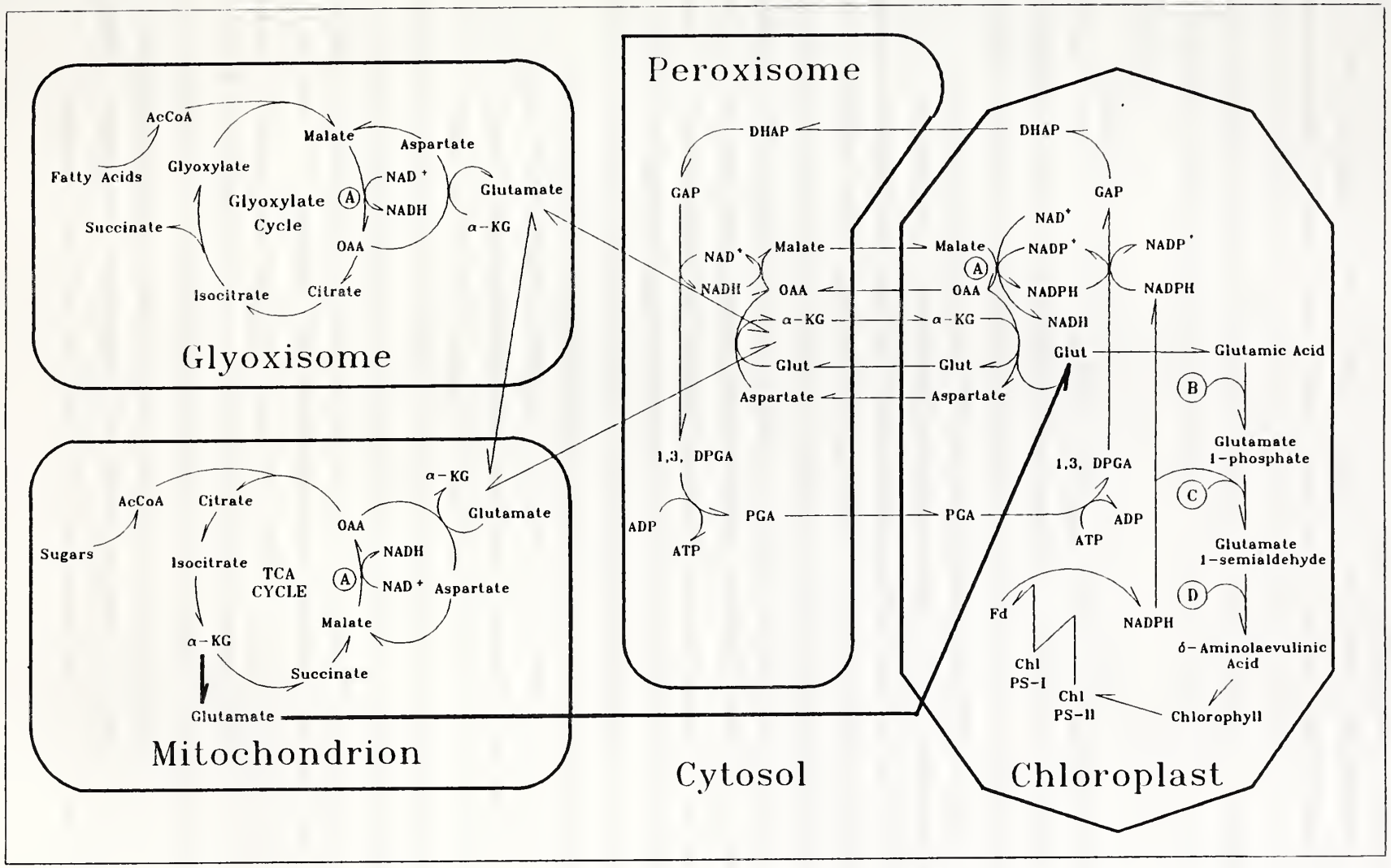


Figure 1. Hypothetical mechanism demonstrating interrelationships among subcellular intermediates involved in the synthesis of chlorophyll in mutant soybean lines. The mechanism shows (A) malate dehydrogenase in the mitochondrion, glyoxisome, and chloroplast as well as (B) glutamate 1-phosphotransferase, (C) glutamate 1-semialdehyde:NADP$^+$ oxidoreductase, and (D) glutamate 1-semialdehyde aminotransferase in the chloroplast.

USDA-ARS-NCAUR
Phytoproducts Research Unit
1815 N. University St.
Peoria, Illinois 61604
and
USDA-ARS
Box 196
Stoneville, MS 38776

Identification of a *Glycine soja* line low in total raffinose

Introduction: Soybean is a rich source of protein and oil. However, mature soybean alos contains up to 6% on a dry weight basis of oligosaccharides of the raffinose family, predominantly stachyose and raffinose. Humans and other monogastric animals, such as poultry and swine, lack the *a* - galactosidase required to hydrolyze these raffinose saccharides. This leads to digestive problems with high soybean diets, including flatulence (Cristofaro et al., 1974) and poor caloric utilization (Muztar and Slinger, 1981). Elimination of raffinose saccharides from soymeal has improved the recovery of metabolizable energy by 25% in poultry feed (Leske et al.,1991). Therefore, production of soybeans low in raffinose saccharides will increase the use and value of soybeans as food and feed.

Hymowitz and Collins (1974) were among the pioneers to determine the variability of sugar content in soybeans and to identify lines that contain low raffinose saccharides. Among 195 G. max, 23 G. gracilis and 12 G. soja of germplasm seeds examined, a few lines were identified to have relatively low raffinose saccharides. However, none of the low trait was heritable. Kuo et al. (1986) were unable to identify any soybean (G. max) among 140 pre-1945 varieties, maturity groups (MG) 000-IV that has low raffinose saccharide content, or any of the pre-1945 G. max in MG V-X analyzed later. Subsequently, screening work was extended to include wild soybean. Herein we report the selection of a G. soja line that contains a low amount of total raffinose and stachyose.

Materials and Methods: In 1987, 475 lines of G. soja were grown in hill plots at Stoneville, MS. Twelve seeds of each line were planted in hills spaced 1.2 m apart in rows spaced 2 m apart. A 2 m cane was placed at each hill to provide support for the viny plants. In order to recover enough seed (for oligosaccharide analyses) before shattering losses occurred, the plants were cut when about 50% of the pods were mature and were placed in a cloth bag until pods dehisced.

Seeds were then separated from plant material and cleaned. All seeds were shipped to the National Center for Agricultural Utilization Research, Peoria, IL for analyses. G. max cultivars 'Sharkey,' 'Tracey-M,' and 'D84-8457' (Tracey-M sb, 1n) were included in the analyses. Twenty lines having relatively low total amounts of raffinose and stachyose, and twenty with high amounts, were planted in the field in 1991 along with sharkey, Tracy-M and D84-8457. Cultural practices and method of analyses for oligosaccharides were the same as those described for the 1987 planting.

Analytical Procedure: Approximately 7 g of wild soybeans were placed in a beaker and dried in a forced air oven for 5 days at 50°C. The seeds were then transferred to a 50-g bottle, sealed and allowed to cool for 1 hour. They were ground in a Varco model MX-228 electric dry-food grinder and returned to the 50-g bottle and moisture in the ground meal was determined. Approximately 3 g of ground meal was used to determined. Approximately 3 g of ground meal was used to determine total oil content by Butt extraction, according to the official AOCS Method (1988). The oil content of G. max cultivars was measured by near-infrared reflectance spectroscopy with suitable calibrations using ground meal for the 1987 seeds or using whole bean for the 1991 crop. Soluble oligosaccharides in the defatted soymeal were analyzed and quantitated by HPLC based on the procedure described by Kuo et al., (1988), except that a Varex model II evaporative light scattering detector was used to monitor the chromatography. The content of each raffinose saccharide was expressed as mg per g dry weight of seed.

Results and DIscussion: Amounts of raffinose, stachyose, and total raffinose + stachyose of selected lines are given in Table 1. Considerable differences occurred in levels of sugars for the same line grown in two different years. However, one line, PI424089, was consistently low in both years. It had a total raffinose + stachyose content of 20.8 mg/g dry seed from the 1987 planting and 18.5 mg/g dry seed in the 1991 planting. The mean for the two years was 14.8 and 4.9 mg/g, or 22.2 and 9.7 μmol/g of dry seed for stachyose and raffinose, respectivley. This is about 30% of the raffinose + stachyose found in three G. max lines. On the basis of these results, the crosses Sharkey x PI 424089 and D84-8457 x PI 424089 were made in 1992. F_2 populations from these crosses were grown in the field in 1993. Seeds from individual F_2 plants have been harvested and will be analyzed for raffinose and stachyose content. The objective is to identify a plant with a low amount of raffinose + stachyose for use as a parent in

backcross to G. max, because all of the approximately 600 F_2 plants were similar to the G. soja plant type.

References:

Cristoforo, E. , F. Mottu and J.J. Wuhrmann. 1974. Involvement of the raffinose family of oligosaccharides in flatulence. In: Sugars in Nutrition, H.L. Sepple and K.W. McNutt. Eds., Academic Press, New York, Chap. 20, pp. 313-336.

Hymowitz, T. and F. I. Collins. 1974. Variability of sugar content in seed of Glycine max (L.) Merrill and G. soja Sieb. and Zucc. Agron. J. 66:239-240.

Kuo, T.M., J. VanMiddlesworth and W.J. Wolf. 1986. Content of soluble oligosaccharides in various plant seeds. Plant Physiol. 80(S):22.

Kuo, T .M., J. VanMiddlesworth and W.J. Wolf. 1988. Content of raffinose oligosaccharides and sucrose in various plant seeds. J. Agric. Food Chem. 36: 32-36.

Leske, K. L., O. Akavanichan, T.K. Chen and C. N. Coon. 1991. Effect of ethanol extract on nitrogen-corrected true metabolizable energy for soybean meal with broilers and rooster. Poultry Sci. 70:892-895 .

Mutzar, A.J. and S.J. Slinger. 1981. A comparison of the true and apparent metablizable energy measures using corn and soybean meal samples. Poultry Sci. 60:611-616.

Official Methods and Recommended Practices of the American Oil Chemists' Society, 3rd Edn., AOCS, Champaign, IL, revised to 1988, Methods Ac 3-44.

T.M. Kuo
T.C. Kilen

USDA, ARS Field Crops Research Unit
IOWA STATE UNIVERSITY
Ames, Iowa 50011

Implications of a nuclear inherited chlorophyll retention mutant ($d_1d_1d_2d_2$) on conditional lethality in soybean, [*Glycine max* (L.) Merr.]

Introduction: Palmer and Cianzio (1985) first noted an interaction leading to conditional lethality in soybean when crossing chlorophyll-deficient cytoplasmically inherited mutant T275 (cyt-Y_2) as female with nuclearly-inherited chlorophyll deficient tan-saddle seed coat mutant T253 as male. Conditional lethality is manifested as death occurring under field conditions but with a capability of survival in the greenhouse under reduced light levels. Hedges and Palmer (1992) and Amberger, Shoemaker, and Palmer (1992) reported that three chlorophyll deficient malate dehydrogenase null mutants, all of which were derived from the progeny of independent germinal revertants of the w_4-mutable (T322) line (Palmer et al, 1989), that were allelic to T253. Analysis of T253 determined that it was malate dehydrogenase null (Hedges and Palmer, 1992). Palmer (1992) made reciprocal crosses of T275 X T253 and T275 X T323 and found that T275, when used as the female parent to either T253 or T323, resulted in conditional lethality in F_2 plants.

Palmer and Minor (in press) tested T253 and T323 with the six remaining maternally inherited chlorophyll deficient mutants (Cianzio and Palmer, 1992) and with maternally inherited cotyledon color mutant cyt-G_1 (Terao, 1918). They found that all six maternally inherited chlorophyll deficient mutants (cyt-Y_3 to cyt-Y_8) along with cyt-Y_2 when used as female parent, elicited the same interaction. The maternally inherited cotyledon color mutant (cyt-G_1) did not cause an interaction. At the same time that the Palmer and Minor study was carried out, T253 and T323 were crossed with a nuclearly inherited cotyledon color mutant ($d_1d_1d_2d_2$) to complete tests of all known chlorophyll anomolies for this type of interaction. We report the results of this study.

Materials and Methods: Genetic stocks L69-4267 ($d_1d_1d_2d_2$) and T323 (CD-1) were crossed reciprocally in winter 1991 at the University of Puerto Rico-Iowa State University Soybean Nursery at Isabella, Puerto Rico. F_1 seeds were collected and planted for seed increase in February 1992 in Puerto Rico. F_1 plants were allowed to mature and were single plant-threshed at harvest. Resulting F_2 seeds were returned to Ames, Iowa. F_2 seeds were scored for

cotyledon color prior to being tested for MDH using starch gel electrophoresis (Cardy and Beversdorf, 1984; Weeden and Wendel, 1989). Seed identity was maintained and seedlings were transplanted to the field at the Bruner Farm, Ames, Iowa. Plants were observed throughout the summer for signs of lethality. F_2 plants were single plant-threshed and cotyledon color of the F_3 seed was scored.

Goodness of fit between observed and expected F_2 ratios for cotyledon color was determined using Chi-square tests.

Results: The F_2 data were 321 yellow seed embryo and 23 green seed embryo, a good fit to the expected 15:1 ratio ($X^2 = 0.11$, $P = 0.74$). 105 yellow seed embryo and all 23 green seed embryo seeds were tested for MDH and the following data was collected:

84 yellow cotyledon, MDH present
21 yellow cotyledon, MDH absent
14 green cotyledon, MDH present
9 green cotyledon, MDH absent

It was expected that this would fit a 9:3:3:1 ratio; however the variation from the expected was significant ($X^2 = 6.67$, $P = 0.08$). The deviation is not due to linkage between either the D_1 locus or the D_2 locus and the Mdh locus because the two parental classes are not in excess, but it may be an artifact of small sample size. No conditional lethality was observed in the F_2 plants. F_3 seed segregated for cotyledon color as expected (data not presented). No interaction was observed in crosses between these two nuclear mutants.

Discussion: The results from this study as well as the Palmer and Minor study (in press) indicated that only cytoplasmically-inherited chlorophyll deficient foliage mutants when used as female parent with T253 and T323 are capable of contributing to the conditionally lethal interaction. Other chlorophyll anomolies whether nuclearly- or maternally-inherited do not evoke this response (Palmer and Mascia 1980).

These data further substantiate that some common factor within the cyt-Y mutants when coupled with chlorophyll-deficient malate dehydrogenase null mutants T253 and T323 cause this interaction.

At present, the physiology behind the conditionally lethal interaction is being studied (Minor unpublished). Earlier speculations centered around the

role of the missing MDH in T253 and T323 in the interation. Recent findings show that perhaps the cyt-Y mutations play a larger role than once believed (Minor unpublished). Further investigation at this point is necessary.

References:

Amberger, L.A., R.C. Shoemaker, and R.G. Palmer. 1992. Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. Theor. Appl. Genet. 84:600-607.

Cardy, B.J., and W.D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes in soybean [Glycine max (L.) Merr.]. Dep. Crop Sci. Tech. Bull. 119/8401. University of Guelph, Ontario, Canada.

Cianzio, S.R., and R.G. Palmer. 1992. Genetics of five cytoplasmically-inherited yellow foliar mutants in soybean. J. Hered. 83:70-72.

Hedges, B.R., and R.G. Palmer. 1992. Inheritance of malate dehydrogenase nulls in soybean. Biochem. Genet. 30:491-502.

Palmer, R.G. 1992. Conditional lethality involving a cytoplasmic mutant and chlorophyll deficient malate dehydrogenase mutants in soybean. Theor. Appl. Genet. 85:389-393.

Palmer, R.G., and S.R. Cianzio. 1985. Conditional lethality involving nuclear and cytoplasmic chlorophyll mutants in soybeans. Theor. Appl.Genet. 70: 349-354.

Palmer, R.G., B.R. Hedges, R.S. Benavente, and R.W. Groose. 1989. w_4-mutable line in soybean. Dev. Genet. 10:542-551.

Palmer, R.G., and P.N. Mascia. 1980. Genetics and ultrastructure of a cytoplasmically-inherited yellow mutant in soybean. Genetics 95:985-1000.

Terao, H. 1918. Maternal inheritance in the soybean. Am. Nat. 52:52-56.

Weeden, N.F., and J.F. Wendel. 1989. Genetics of plant isozymes. In D.F. Soltis and P.S. Soltis (eds.), Isozymes in Plant Biology, pp 46-72. Dioscorides Press, Portland, OR.

V. Christine Minor

R. G. Palmer - USDA

UNIVERSITY OF MINNESOTA,
Los Andes,Chile
and
UNIVERSITY OF UTAH

Recombinant Inbred Line Population from the Cross Minsoy x Noir 1

At the 1994 Soybean Breeders Workshop, L.M. Mansur discussed the usefulness of recombinant inbred (RI) lines in mapping traits in soybeans. In particular, the RI population from the cross of `Minsoy' (PI 27890) and Noir 1 (PI 290136) was described. RI lines are unique genetic materials because the population can be propagated indefinitely. They are particularly useful for quantitative trait loci (QTL) mapping. In large RI populations, linked genes have a greater probability of recombination. Because RI are homozygous, DNA for mapping is never exhausted since more plants with the same genotype can be grown repeatedly. These lines can be evaluated for any trait that has phenotypic variability in the population and evaluation can be done over several years and/or locations by many different researchers simultaneously. The information collected on such traits can be accumulated and related to an RFLP genetic database for the RIL. Thus the RIL serve as a focal point for the deposit of phenotypic data and QTL mapping information that may come from many scientists. Over time, the RIL population increases in its value to the scientific community because of the correlation of accumulated information and the continued availability of the genetic material.

The Minsoy x Noir 1 population consists of more than 250 RIL which are currently in the F12 generation. It was developed by L. Mansur from F2 seed supplied by Dr. Reid Palmer, USDA/ARS, Iowa State University. It has been evaluated for the simply inherited traits flower, seed coat, and hilum color; peroxidase activity, pubescence tip shape and root florescence; and for the quantitative traits date to flower (R1) and maturity (R8); plant height and lodging; leaf length, width and area; seed size, protein, oil content and yield. To date, 200 RFLP markers have been placed on the map by screening 225 RI lines.

We believe this is a valuable public resource and plan to make the database available to all who are interested in using the information. Seed of the ca 250 RIL also will be available to those who wish to collaborate. The RFLP database is currently maintained in the laboratory of K.G. Lark. We do ask that those who receive seed and/or use the database agree to make their data publicly available and share it in a timely fashion in the appropriate manner. Seed requests from U.S. scientists can be directed to J.H. Orf, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108. Seed requests from international scientists can be directed to L.M. Mansur, Mansur Agricultural Services, P.O. Box 520, Los Andes, CHILE. Initially the parents and 50 random RI lines will be sent to researchers so that

they can assess genetic variability for the trait of interest. The database information can be requested from K.G. Lark, Department of Biology, University of Utah, Salt Lake City, UT 84112.

In addition to providing obvious opportunities for new soybean research, we hope that this resource will foster new and useful collaborations between members of the soybean community. Preliminary reports of data with the RI population have been presented at the Agronomy Meetings 1993, the Latin American Biotechnology Congress 1993, and at the Genome II meeting 1994. One paper using this database is published (Mansur et al., 1993, TAG), another is in press (Lark et al., 1994, TAG), and two others are under review in Crop Science and Genetics.

References:

Mansur, L.M., J.H. Orf, and K.G. Lark. 1993. Determining linkage of quantitative trait loci to RFLP markers using extreme phenotypes of recombinant inbreds of soybean [Glycine max (L.) Merr.]. Theor. Appl. Gen. 86:914-918.

Mansur, L.M., J.H. Orf, and K.G. Lark. 1993. Correlations for yield and yield-determining traits for soybeans grown in the U.S. and Chile. Agron. Abs., p. 93.

Mansur, L.M., J. Orf, and K.G. Lark. 1993. Un nuevo método para detectar ligamento entres sondas RFLP y loci de caracteres métricos usando DNA obtenido de fenotipos extremos de lineas recombinantes homozigotas., p. 159, 3er Congreso Latinoamericano y Nacional de Biotecnologia, Universidad de Chile, Santiago, Chile, Nov. 16-19, 1993.

Lark, K.G., J. Orf, and L.M. Mansur. 1994. Epistatic expression of quantitative trait loci (QTL) in soybeans [Glycine max (L.) Merr.] determined by QTL association with RFLP alleles. Theor. Appl. Gen. 87: (in press).

J.H. Orf
L.M. Mansur
K.G. Lark

UNIVERSITY OF MISSOURI
117 Curtis Hall
Columbia, Missouri 65211
and
PIONEER HI-BRED INTL., INC.
Plant Breeding Division
Johnston, Iowa 50131-1004

DNA Fingerprinting of Soybean Accessions with Resistance to Soybean Cyst Nematode

Introduction: Soybean cyst nematode (SCN), Heterodera glycines Ichinohe is the most prominent cyst nematode in the United States and occurs worldwide on soybean. This pest causes tremendous yield losses in soybean producing states every year. In the U.S. an estimated percent loss caused by SCN was 3.0% (Sciumbiato, 1993).

Crop losses due to SCN have been reduced predominantly by the use of host plant resistance. Over 35 soybean accessions with host resistance to different SCN populations and to their isolates have been identified. Nearly all SCN-resistant cultivars in the U.S. have been bred using 'Peking' and/or PI88788 as genetic resources for host plant resistance. The resistance in cultivars has not been durable because of the emergence of new races of SCN to which these cultivars are susceptible. Genetic diversity and/or gene pyramiding of SCN-resistance genes in cultivars will produce durable resistance, but traditional technologies do not possess the tools for precise gene identification. As a first step, we are attempting to determine the genetic relationships among 29 soybean accessions resistant to SCN using RFLP analysis for developing appropriate mapping populations and preliminary results have been presented here on fingerprinting.

Materials and Methods: Seedlings from 29 soybean accessions with SCN-resistance were grown in the greenhouse for DNA extraction. These were obtained from USDA-ARS southern and northern soybean germplasm collections (courtesy of Dr.s Nelson, Bernard and Hartwig). Two susceptible controls cv. Essex and cv. Hutchison were also included in the study. These resistant accessions represent a stratified sample from five conutries: Argentina, China, Japan, Russia, and South Korea.

Total genomic DNA extracts were obtained using CTAB methods. Purified DNA was digested individually with restriction endonuclease Dra 1, Eco RV, Hind III and Taq 1, and then size frationated in 0.8% agarose gels and souther blotted to nylon membrane. Random primer generated DNA probes were used for hybridization of genomic DNA. The genomic probes were provided by Dr.s R.C. Shoemaker - USDA-ARS-FCR, Ames, IA and David Webb, Pioneer Hi-Bred Intl. Inc., Johnston, IA. Hybridizations and washes were conducted in glass bottles at 65°C using hybridization incubator (Robbins Scientific Co., CA). Membranes were exposed to Kodak X-oat AR film for 48-96 hours.

The DNA fingerprinting technique includes marker designations, map locations, preferred restriction enzymes, band sizes and scores, and a polymorphism index. The polymorphism index for each probe was calculated using the equation $1-\sum\sum pi^2$, where p= the allele frequency for i alleles 1 to N.

All bands having equivalent migration distance were given the same letter score. Genetic distances among all possible pairs of accessions were estimated from a modification of Nei's similarity equation (Nei and Li, 1979) as used by Keim et al., (1992) in soybean.

The genetic distances between all pairs of accessions and varieties estimated with RFLP information (GD_R). The proportion of similar RFLP loci, S_{XY}, between pairs of PI lines was estimated as $2N_{XY}(N_X+N_Y)$ where N_{XY} is the number of RFLP loci for which PI lines X and Y possess the same allele, N_X is the number of alleles identified in line X and N_Y is the number of alleles identified in line Y. GD_R was calculated as $1-S_{XY}$. Based on the GD_R matrix, a tentative dendogram was generated (fig.1) to graphically display the calculated distances between genotypes.

Results and Discussion: In this preliminary observation, a limited number of probes were evaluated. Of the 22 probes examined,13 were polymorphic producing between 2-9 banding patterns. We found that 60% of the probes detected variation among the 29 resistant and two susceptible genotypes for SCN reaction. The probe polymorphism frequency was 1.7. Preliminary estimates of the genetic distance between all pairs of the genotypes were given in Table 1.

GD_R is a direct measure of the porportion of RFLP loci that are different. Significant among these observations are the two susceptible cultivars which are very different from most of the other accessions. The distance analysis showed higher values that separate 'Peking' from 'PI88788' and 'PI404166'. All three of them are resistant to SCN Race 3. Our previous genetic studies also have shown

that 'Peking' and 'PI 88788' have different loci for SCN resistance (Rao-Arelli and Anand, 1988; Rao-Arelli et al., 1992). Additional studies also indicated that soybean accessions 'Peking', 'PI90763' and 'PI404166' carried at least one gene that is different among them for Race 5 resistance (Anand and Rao-Arelli, 1989).

Genetic distance is higher that separates 'PI88788' from ' PI89772'. Similarly, 'PI437654' is separated from 'PI438489B', 'PI897772', and 'PI404166' based on higher genetic distance values. This is supported by our previous genetic findings that gave segregation in F_2 generation when 'PI437654' was crossed to each of the three PI lines '438489B', '89772' and '404166'. This has indicated that different loci conditioned SCN resistance to Races 3 and 5 in these parental lines (Rao-Arelli et al., 1991).

Genetic distances are also higher in PI lines '398680', 399061', '437655', and '84751' and '89772', which indicated higher genetic variation among these accessions, thereby showing the degree of genetic unrelatedness in their evolution.

In the absence of typical pedigree information for accessions, fingerprinting should be most useful in determining their genetic relationship to developing populations for gene mapping studies. Currently, more markers are being developed which can discriminate between resistant and susceptible genotypes also among resistant accessions.

References:

Anand, S.C., and A.P. Rao-Arelli. 1989. Genetic analyses of soybean genotypes resistant to soybean cyst nematode race 5. Crop Sci. 28:1181-1186.

Keim, P., W. Beavis, J. Schupp, and R. Freestone. 1992. Evaluation of soybean RFLP marker diversity in adapted germplasm. Theor. Appl. Genet. 85:205-212.

Nei, M., and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USDA 76:5256-5273.

Rao-Arelli; A.P., and S.C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci. 28:650-652.

Rao-Arelli, A.P. S.C. Anand and J.A. Wrather. 1991. Genetic relationship of resistance to soybean cyst nematode races 3 and 5 in soybean accessions. Agronomy Abst. Amer. Soc. Agron. Madison, WI p.113.

Rao-Arelli, A.P., S.C. Anand, and J.A. Wrather. 1992. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Sci. 32:862-866.

Sciumbato, G.L. 1993. Soybean disease loss estimates for the Southern United States during 1988-1991. Plant Dis. 77:954-956.

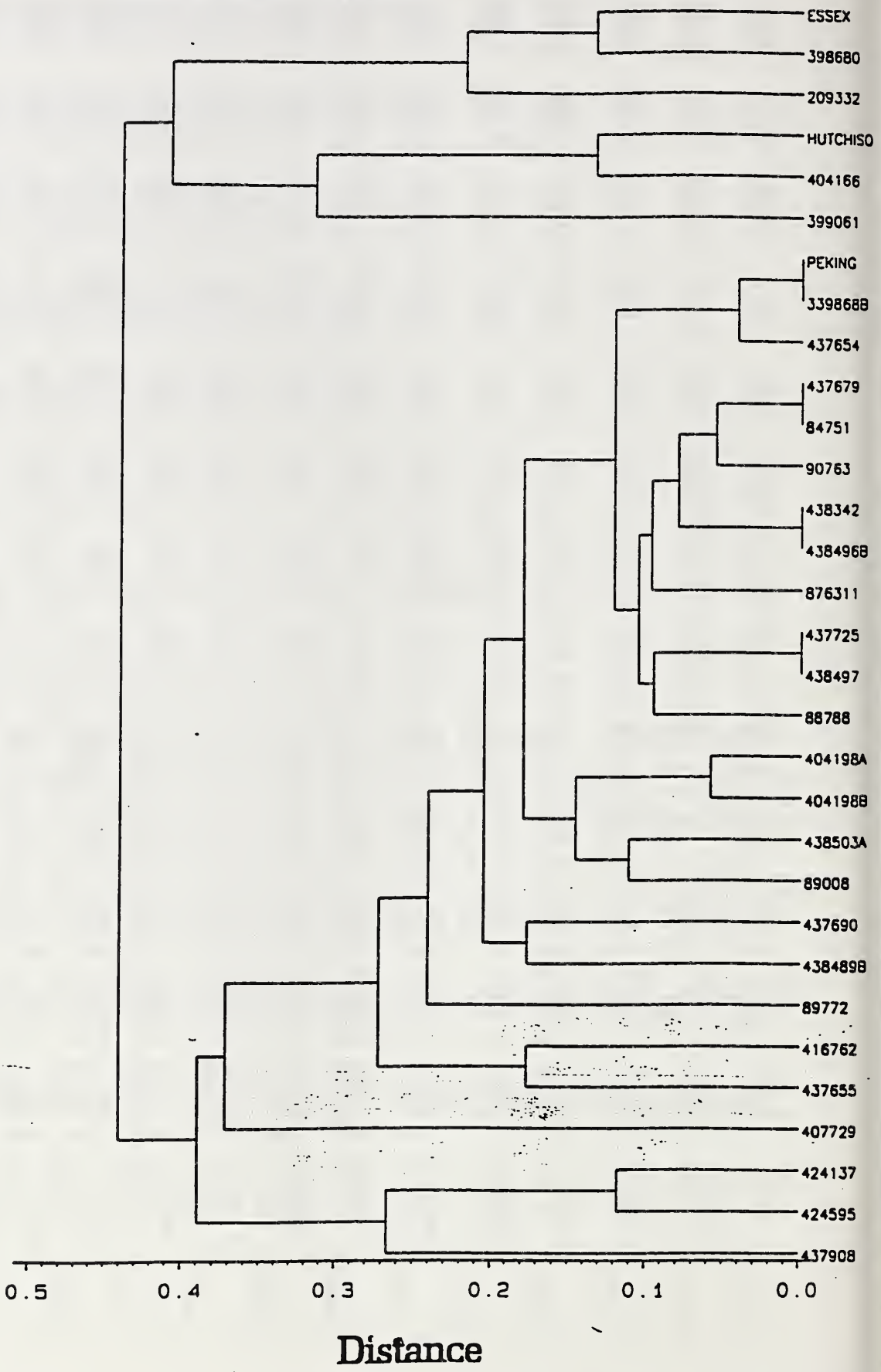
A.P. Rao-Arelli
G.O. Myers
D.M. Webb

Table 1. Estimates of the genetic distances between all pairs of genotypes.

NEIDST	209332	339868B	398680	399061	404166	404198A	404198B	407729	416762	424137	424595	437654	437655
:	437679	437690	437725	437908	438342	438489B	438496B	438497	438503A	84751	876311	88788	89008
:	89772	90763	ESSEX	HUTCHISO	PEKING								
209332	0.000	0.353	0.235	0.529	0.471	0.235	0.235	0.353	0.294	0.588	0.529	0.412	0.235
:	0.353	0.353	0.400	0.353	0.294	0.353	0.294	0.412	0.235	0.353	0.412	0.412	0.333
:	0.412	0.389	0.200	0.400	0.286								
339868B	0.353	0.000	0.588	0.412	0.353	0.118	0.176	0.353	0.235	0.412	0.353	0.059	0.294
:	0.059	0.118	0.133	0.294	0.118	0.176	0.118	0.118	0.118	0.059	0.118	0.118	0.111
:	0.235	0.111	0.400	0.400	0.000								
398680	0.235	0.588	0.000	0.412	0.471	0.471	0.471	0.471	0.412	0.471	0.471	0.647	0.471
:	0.588	0.471	0.467	0.353	0.529	0.588	0.529	0.529	0.471	0.588	0.529	0.529	0.444
:	0.647	0.611	0.133	0.267	0.571								
399061	0.529	0.412	0.412	0.000	0.294	0.412	0.471	0.647	0.529	0.353	0.353	0.471	0.706
:	0.471	0.529	0.400	0.294	0.412	0.588	0.412	0.412	0.294	0.471	0.529	0.412	0.333
:	0.647	0.500	0.533	0.333	0.429								
404166	0.471	0.353	0.471	0.294	0.000	0.471	0.471	0.353	0.529	0.588	0.529	0.412	0.588
:	0.353	0.353	0.333	0.353	0.412	0.471	0.412	0.294	0.353	0.353	0.294	0.412	0.389
:	0.529	0.389	0.267	0.133	0.286								
404198A	0.235	0.118	0.471	0.412	0.471	0.000	0.059	0.353	0.235	0.412	0.353	0.176	0.294
:	0.176	0.118	0.267	0.294	0.118	0.176	0.118	0.235	0.118	0.176	0.235	0.235	0.111
:	0.235	0.222	0.400	0.533	0.143								
404198B	0.235	0.176	0.471	0.471	0.471	0.059	0.000	0.353	0.176	0.412	0.353	0.235	0.235
:	0.118	0.176	0.200	0.294	0.059	0.176	0.059	0.176	0.176	0.118	0.176	0.176	0.167
:	0.176	0.167	0.400	0.533	0.143								
407729	0.353	0.353	0.471	0.647	0.353	0.353	0.353	0.000	0.412	0.647	0.647	0.412	0.471
:	0.353	0.235	0.467	0.471	0.412	0.353	0.412	0.412	0.353	0.353	0.294	0.412	0.389
:	0.294	0.333	0.267	0.400	0.286								
416762	0.294	0.235	0.412	0.529	0.529	0.235	0.176	0.412	0.000	0.471	0.412	0.294	0.176
:	0.176	0.235	0.267	0.353	0.235	0.235	0.235	0.235	0.353	0.176	0.235	0.235	0.278
:	0.235	0.222	0.333	0.333	0.214								
424137	0.588	0.412	0.471	0.353	0.588	0.412	0.412	0.647	0.471	0.000	0.118	0.353	0.647
:	0.412	0.529	0.333	0.294	0.353	0.353	0.353	0.353	0.412	0.412	0.471	0.353	0.333
:	0.588	0.444	0.600	0.533	0.357								
424595	0.529	0.353	0.471	0.353	0.529	0.353	0.353	0.647	0.412	0.118	0.000	0.294	0.588
:	0.353	0.471	0.267	0.235	0.294	0.294	0.294	0.294	0.353	0.353	0.412	0.353	0.278
:	0.529	0.389	0.533	0.467	0.286								
437654	0.412	0.059	0.647	0.471	0.412	0.176	0.235	0.412	0.294	0.353	0.294	0.000	0.353
:	0.118	0.176	0.200	0.353	0.176	0.118	0.176	0.176	0.176	0.118	0.176	0.176	0.167
:	0.294	0.167	0.467	0.467	0.000								

437655	0.235	0.294	0.471	0.706	0.588	0.294	0.235	0.471	0.176	0.647	0.588	0.353	0.000
	0.235	0.294	0.267	0.529	0.294	0.294	0.294	0.294	0.412	0.235	0.294	0.294	0.389
	0.294	0.278	0.333	0.533	0.286								
437679	0.353	0.059	0.588	0.471	0.353	0.176	0.118	0.353	0.176	0.412	0.353	0.118	0.235
	0.000	0.176	0.067	0.294	0.059	0.176	0.059	0.059	0.176	0.000	0.059	0.059	0.167
	0.176	0.056	0.400	0.400	0.000								
437690	0.353	0.118	0.471	0.529	0.353	0.118	0.176	0.235	0.235	0.529	0.471	0.176	0.294
	0.176	0.000	0.267	0.412	0.235	0.176	0.235	0.235	0.235	0.176	0.118	0.235	0.167
	0.235	0.222	0.267	0.400	0.143								
437725	0.400	0.133	0.467	0.400	0.333	0.267	0.200	0.467	0.267	0.333	0.267	0.200	0.267
	0.067	0.267	0.000	0.267	0.133	0.267	0.133	0.000	0.267	0.067	0.133	0.067	0.250
	0.267	0.125	0.333	0.385	0.083								
437908	0.353	0.294	0.353	0.294	0.353	0.294	0.294	0.471	0.353	0.294	0.235	0.353	0.529
	0.294	0.412	0.267	0.000	0.235	0.412	0.235	0.235	0.235	0.294	0.353	0.353	0.222
	0.471	0.333	0.400	0.267	0.214								
438342	0.294	0.118	0.529	0.412	0.412	0.118	0.059	0.412	0.235	0.353	0.294	0.176	0.294
	0.059	0.235	0.133	0.235	0.000	0.235	0.000	0.118	0.118	0.059	0.118	0.118	0.111
	0.235	0.111	0.467	0.467	0.071								
438489B	0.353	0.176	0.588	0.588	0.471	0.176	0.176	0.353	0.235	0.353	0.294	0.118	0.294
	0.176	0.176	0.267	0.412	0.235	0.000	0.235	0.235	0.294	0.176	0.235	0.235	0.278
	0.235	0.222	0.400	0.533	0.071								
438496B	0.294	0.118	0.529	0.412	0.412	0.118	0.059	0.412	0.235	0.353	0.294	0.176	0.294
	0.059	0.235	0.133	0.235	0.000	0.235	0.000	0.118	0.118	0.059	0.118	0.118	0.111
	0.235	0.111	0.467	0.467	0.071								
438497	0.412	0.118	0.529	0.412	0.294	0.235	0.176	0.412	0.235	0.353	0.294	0.176	0.294
	0.059	0.235	0.000	0.235	0.118	0.235	0.118	0.000	0.235	0.059	0.118	0.118	0.222
	0.235	0.111	0.333	0.333	0.071								
438503A	0.235	0.118	0.471	0.294	0.353	0.118	0.176	0.353	0.353	0.412	0.353	0.176	0.412
	0.176	0.235	0.267	0.235	0.118	0.294	0.118	0.235	0.000	0.176	0.235	0.235	0.111
	0.353	0.222	0.400	0.400	0.071								
84751	0.353	0.059	0.588	0.471	0.353	0.176	0.118	0.353	0.176	0.412	0.353	0.118	0.235
	0.000	0.176	0.067	0.294	0.059	0.176	0.059	0.059	0.176	0.000	0.059	0.059	0.167
	0.176	0.056	0.400	0.400	0.000								
876311	0.412	0.118	0.529	0.529	0.294	0.235	0.176	0.294	0.235	0.471	0.412	0.176	0.294
	0.059	0.118	0.133	0.353	0.118	0.235	0.118	0.118	0.235	0.059	0.000	0.118	0.167
	0.235	0.111	0.333	0.333	0.071								
88788	0.412	0.118	0.529	0.412	0.412	0.235	0.176	0.412	0.235	0.353	0.353	0.176	0.294
	0.059	0.235	0.067	0.353	0.118	0.235	0.118	0.118	0.235	0.059	0.118	0.000	0.222
	0.235	0.111	0.400	0.467	0.071								
89008	0.333	0.111	0.444	0.333	0.389	0.111	0.167	0.389	0.278	0.333	0.278	0.167	0.389
	0.167	0.167	0.250	0.222	0.111	0.278	0.111	0.222	0.111	0.167	0.167	0.222	0.000
	0.333	0.211	0.438	0.375	0.133								
89772	0.412	0.235	0.647	0.647	0.529	0.235	0.176	0.294	0.235	0.588	0.529	0.294	0.294

Fig 1. Dendrogram showing genetic relationships among genotypes based on genetic distances.



IOWA STATE UNIVERSITY
Department of Zoology/Genetics &
Department of Agronomy
Ames, IA 50011

Development of near-isogenic lines for root fluorescence

Root fluorescence in soybean is a phenomenon in which roots of seedlings fluoresce when irradiated with ultraviolet light. In a survey of 572 Glycine max (L.) Merr. accessions for root fluorescence, Delannay and Palmer (1982), identified four loci controlling root fluorescence. Three of the mutant loci were recessive, fr1, fr2, or fr4 and one mutant locus was dominant, Fr3. Geographical distribution of the four genes was unequal; fr1 was found in accessions from all geographical areas, Fr3 and fr4 were found only in accessions from Asia, and fr2 was restricted to accessions from Europe.

Sawada and Palmer (1987) obtained 13 non-fluorescent mutants from 154,016 seedlings derived from soybean lines treated with six mutagens. In addition to recovering alleles for non-fluorescent roots at the fr1, fr2, or fr4 loci, a non-allelic mutant, fr5, was identified.

The objective of this study was to develop near-isogenic lines in the cultivar Hark that contained the fr1, fr2, Fr3, or fr4 mutations. Single and double mutants were produced, which resulted in six pairs of near-isogenic lines. The fr5 allele is in the cultivar Williams and was not used in this study.

Development of the near-isogenic lines with fr1, fr2, or fr4:

The four standard mutant lines for non-fluorescent roots were:

Line	Gene
Minsoy	fr1 fr1
PI 290136	fr2 fr2
PI 424078	Fr3 Fr3
PI 404165	fr4 fr4

The recurrent line was the cultivar Hark (Weber 1967), Fr1 Fr1 Fr2 Fr2 fr3 fr3 Fr4 Fr4. The development of the independent near-isogenic lines of Hark for each of the three loci fr1 fr1, fr2 fr2, or fr4 fr4 was similar to that shown below. The general scheme was:

Development of individual near-isogenic lines for the fr1, fr2, or fr4 loci:

	Parents	
Generation	Female Male	Comments
	Minsoy (fr1 fr1) Hark (Fr1 Fr1)	
F1	Fr1 fr1	
F2	1 Fr1 Fr1 : 2 Fr1 fr1 : 1 fr1 fr1*	* selected one non-fluorescent root plant
BC1	Hark (Fr1 Fr1) x F2 (fr1 fr1)	
BC1 F1	Fr1 fr1	
BC2	Hark (Fr1 Fr1) x BC1F1 Fr1 fr1	- obtained a minimum of 8 F1 seeds
BC2F1	1 Fr1 Fr1 1 Fr1 fr1	- used 8 different BC2F1 plants to generate BC3F1 plants - root fluorescence was checked from selfed seeds from each of the 8 BC2F1 plants
BC3	Hark (Fr1 Fr1) x BC2F1 Fr1 fr1	- BC3F1 seed from a BC2F1 plant segregating for root fluorescence was used as a male parent - obtained a minimum of 8 F1 seeds
BC3F1	1 Fr1 Fr1 1 Fr1 fr1	
BC4 " " "	Hark (Fr1 Fr1) x BC3F1 Fr1 fr1 " " "	- same as for BC3 " " "
BC6 F1	1 Fr1 Fr1 1 Fr1 fr1	- selected one plant that was Fr1 fr1, based upon progeny testing

A single fr1 fr1 plant was identified and allowed to self pollinate; this line became Hark fr1 fr1. Based upon progeny testing, a single Fr1 Fr1 plant was identified and selfed; this line became Hark Fr1 Fr1. In summary, one BC6F2 plant of genotype fr1 fr1 and one BC6F2 plant of genotype Fr1 Fr1 was saved and became the near-isogenic pair of lines

in Hark. This procedure was repeated for the fr2 and fr4 loci.

Gene pyramiding: The scheme for the development of independent near-isogenic lines of the double mutants of Hark for fr1 fr1 fr2 fr2, fr1 fr1 fr4 fr4, or fr2 fr2 fr4 fr4 is shown below.

Pyramiding of pairs of loci:

Generation	Parents	Comments
	Female Male	
	Hark fr1 fr1 Fr2 Fr2 Hark Fr1 Fr1 fr2 fr2	
F1	Hark Fr1 fr1 Fr2 fr2	
F2	9 Fr1 - Fr2 -	- fluorescent roots--DISCARD
	1 Fr1 Fr1 fr2 fr2	- non-fluorescent roots--SAVE
	2 Fr1 fr1 fr2 fr2	"
	1 fr1 fr1 Fr2 Fr2	"
	2 fr1 fr1 Fr2 fr2	"
	1 fr1 fr1 fr2 fr2*	"
		* Desired genotype (14.3% of non-fluorescent phenotypic class)
F3	From self-pollination of F2 non-fluorescent root plants	
	3 Fr1 Fr1 fr2 fr2	- non-fluorescent roots
	2 Fr1 fr1 fr2 fr2	"
	4 fr1 fr1 fr2 fr2*	"
	2 fr1 fr1 Fr2 fr2	"
	3 fr1 fr1 Fr2 Fr2	"
		* Desired genotype (28.6% of non-fluorescent phenotypic class)
F4	From self-pollination of F3 non-fluorescent root plants	
	7 Fr1 Fr1 fr2 fr2	- non-fluorescent roots
	2 Fr1 fr1 fr2 fr2	"
	10 fr1 fr1 fr2 fr2*	"
	2 fr1 fr1 Fr2 fr2	"
	7 fr1 fr1 Fr2 Fr2	"
		* Desired genotype (35.7% of non-fluorescent phenotypic class)

Verification of double homozygous recessive genotype: Nine F4 plants (non-fluorescent roots) from each of the three putative genotypic combinations (fr1 fr1 fr2 fr2, fr1 fr1 fr4 fr4, and fr2 fr2 fr4 fr4) were used as male parents. Female parents were the appropriate testers; Hark fr1 fr1, Hark fr2 fr2, or Hark fr4 fr4. A minimum of eight hybrid seeds from each combination was obtained.

	Male	Female	
F4	Testcross	Hark fr1 fr1 Fr2 Fr2	Hark Fr1 Fr1 fr2 fr2
	7 Fr1 Fr1 fr2 fr2	all fluorescent	all non-fluorescent
	2 Fr1 fr1 fr2 fr2	1 fluorescent: 1 non-fluorescent	all non-fluorescent
	10 fr1 fr1 fr2 fr2*	all non-fluorescent	all non-fluorescent
	2 fr1 fr1 Fr2 fr2	all non-fluorescent	1 fluorescent: 1 non-fluorescent
	7 fr1 fr1 Fr2 Fr2	all non-fluorescent	all fluorescent
*Desired genotype			

All three double mutants were obtained. One plant of the desired genotype from each combination was selected and seeds were increased.

Development of the near-isogenic lines for the Fr3 locus: The development of near-isogenic lines of Hark for the dominant Fr3 Fr3 locus differed from that used for the recessive Hark near-isogenic lines, fr1 fr1, fr2 fr2, and fr4 fr4. The procedure for the Fr3 lines is shown below.

	Parents	
Generation	Female Male	Comments
	PI 424078 (Fr3 Fr3) Hark (fr3 fr3)	
F1	Fr3 fr3	
F2	1 Fr3 Fr3* : 2 Fr3 fr3 : 1 fr3 fr3	* Desired genotype - selected 5 F2 non-fluorescent plants - root fluorescence was checked from selfed seed from each of the 5 F2 plants
BC1	Hark (fr3 fr3) x F2 (Fr3 Fr3)	
BC1F1	Fr3 fr3	
BC2	Hark (fr3 fr3) x BC1F1 Fr3 fr3	- obtained a minimum of 8 F1 seed
BC2F1	1 Fr3 fr3 1 fr3 fr3	- used 5 different BC2F1 plants to generate BC3F1 plants - root fluorescence was checked from selfed seeds from each of the 5 BC2 F1 plants
BC3	Hark (fr3 fr3) x BC2F1 Fr3 fr3	
BC3F1	1 Fr3 fr3 1 fr3 fr3	
BC4 ▪ ▪ ▪	Hark (fr3 fr3) x BC3F1 Fr3 fr3 ▪ ▪ ▪	- obtained a minimum of 8 F1 seeds
BC6F1	1 Fr3 fr3 1 fr3 fr3	- selected one plant that was Fr3 fr3, based upon progeny testing

A single fr3 fr3 plant was identified and allowed to self pollinate; this line became Hark fr3 fr3. Based upon progeny testing, a single Fr3 Fr3 plant was identified and selfed; this line became Fr3 Fr3. In summary, one BC6F2 plant of genotype fr3 fr3 and one BC6F2 plant of genotype Fr3 Fr3 was saved and became the near-isogenic pair of lines in Hark.

The general scheme for gene pyramiding: The development of the near-isogenic lines of the double mutants of Hark, for Fr3 Fr3 fr1 fr1, Fr3 Fr3 fr2 fr2, or Fr3 Fr3 fr4 fr4 is shown below.

Pyramiding of pairs of loci:

Generation	Parents		Comments
	Female	Male	
	Hark Fr3 Fr3 Fr1 Fr1	Hark fr3 fr3 fr1 fr1	
F1	Hark Fr3 fr3 Fr1 fr1		
F2	1	Fr3 Fr3 Fr1 Fr1	- non-fluorescent roots
	2	Fr3 fr3 Fr1 Fr1	- non-fluorescent roots
	2	Fr3 Fr3 Fr1 fr1	- non-fluorescent roots
	4	Fr3 fr3 Fr1 fr1	- non-fluorescent roots
	1	fr3 fr3 Fr1 Fr1	- fluorescent roots--DISCARD
	2	fr3 fr3 Fr1 fr1	- fluorescent roots--DISCARD
	1	Fr3 Fr3 fr1 fr1*	- non-fluorescent roots
	2	Fr3 fr3 fr1 fr1	- non-fluorescent roots
	1	fr3 fr3 fr1 fr1	- non-fluorescent roots * Desired genotype (7.7% of non-fluorescent phenotypic class)
F3	From self-pollination of F2 non-fluorescent root plants		
	9	Fr3 Fr3 Fr1 Fr1	- non-fluorescent roots
	6	Fr3 fr3 Fr1 Fr1	- non-fluorescent roots
		fr3 fr3 Fr1 Fr1	- fluorescent roots--DISCARD
	6	Fr3 Fr3 Fr1 fr1	- non-fluorescent roots
	9	Fr3 Fr3 fr1 fr1*	- non-fluorescent roots
	6	Fr3 fr3 fr1 fr1	- non-fluorescent roots
	7	fr3 fr3 fr1 fr1	- non-fluorescent roots
	4	Fr3 fr3 Fr1 fr1	- non-fluorescent roots
		fr3 fr3 Fr1 fr1	- fluorescent roots--DISCARD * Desired genotype (19.1% of non-fluorescent phenotypic class)
F4	From self-pollination of F3 non-fluorescent root plants		
	49	Fr3 Fr3 Fr1 Fr1	- non-fluorescent roots
	14	Fr3 fr3 Fr1 Fr1	- non-fluorescent roots
		fr3 fr3 Fr1 Fr1	- fluorescent roots--DISCARD
	14	Fr3 Fr3 Fr1 fr1	- non-fluorescent roots
	49	Fr3 Fr3 fr1 fr1*	- non-fluorescent roots
	14	Fr3 fr3 fr1 fr1	- non-fluorescent roots
	35	fr3 fr3 fr1 fr1	- non-fluorescent roots
	4	Fr3 fr3 Fr1 fr1	- non-fluorescent roots
		fr3 fr3 Fr1 fr1	- fluorescent roots--DISCARD * Desired genotype (27.4% of non-fluorescent phenotypic class)

Verification of double homozygous genotype: Testcrosses

	Male		Female	
F4	Testcross		Hark fr3 fr3 fr1 fr1	Hark Fr3 Fr3 Fr1 Fr1
	49	Fr3 Fr3 Fr1 Fr1	all non-fluorescent[a]**	all non-fluorescent[b]
	14	Fr3 fr3 Fr1 Fr1	1 non-fluorescent: 1 fluorescent	all non-fluorescent
	14	Fr3 Fr3 Fr1 fr1	all non-fluorescent[c]	all non-fluorescent[d]
	49	Fr3 Fr3 fr1 fr1*	all non-fluorescent[e]	all non-fluorescent[f]
	14	Fr3 fr3 fr1 fr1	all non-fluorescent[g]	all non-fluorescent[h]
	35	fr3 fr3 fr1 fr1	all non-fluorescent[i]	all non-fluorescent[j]
	4	Fr3 fr3 Fr1 fr1	3 non-fluorescent: 1 fluorescent	all non-fluorescent

*Desired genotype
**a to j are code numbers for Table; Verification of double homozygous genotype:Self-pollination of testcrosses (see next page).

Nine F4 plants (non-fluorescent roots) from each of the three putative genotypic combinations (Fr3 Fr3 fr1 fr1, Fr3 Fr3 fr2 fr2, and Fr3 Fr3 fr4 fr4) were used as male parents. Female parents were the appropriate testers; Hark fr1 fr1, Hark fr2 fr2, Hark Fr3 Fr3, or Hark fr4 fr4. A minimum of eight hybrid seeds from each combination was obtained.

Based upon testcross data, however, we could not distinguish the desired genotype Fr3 Fr3 fr1 fr1 from several other genotypes which produce the non-fluorescent root phenotype. However, by classifying the self-pollinated progeny of all non-fluorescent testcross plants, we can accurately identify the genotype Fr3 Fr3 fr1 fr1.

Identification of Fr3 Fr3 fr1 fr1 by examination of root fluorescent of self-pollination of non-fluorescent testcross plants:

Verification of double homozygous genotype: Self-pollination of testcrosses

Code numbers from testcrosses	Phenotype
a	13 non-fluorescent:3 fluorescent
b	all non-fluorescent
c	13 non-fluorescent:3 fluorescent all non-fluorescent
d	all non-fluorescent
e*	all non-fluorescent*
f*	all non-fluorescent*
g	all non-fluorescent
h	13 non-fluorescent:3 fluorescent all non-fluorescent
i	all non-fluorescent
j	13 non-fluorescent:3 fluorescent
*Desired genotype Fr3 Fr3 fr1 fr1	

The desired genotype Fr3 Fr3 fr1 fr1 is easily and accurately identified. Self-pollinated progeny of the testcrosses gave classes where all cross combinations, except one cross combination, resulted in segregation for non-fluorescent:fluorescent roots. The exception, all non-fluorescent roots identified the genotype Fr3 Fr3 fr1 fr1.

The transfer of the Fr3 allele to Hark and the subsequent gene pyramiding of the Fr3 allele was facilitated by the linkage of acid phosphatase (Ap) with the Fr3 locus. We had calculated 5.0 ± 2.1 recombination between Ap and Fr3. The linkage data will be presented separately (Palmer, unpublished).

Summary: We have successfully developed the single-gene near-isogenic root fluorescence lines in the cultivar Hark for the fr1 fr1, fr2 fr2, Fr3 Fr3, or fr4 fr4 loci. Also, from the appropriate crosses between pairs of the single mutant near-isogenic Hark lines, double mutants containing the Fr3 Fr3 fr1 fr1, Fr3 Fr3 fr2 fr2, and Fr3 Fr3 fr4 fr4 loci were identified.

References:

Delannay, X. and R. G. Palmer. 1982. Four genes controlling root fluorescence in soybean. Crop Sci. 22:278-281.

Sawada, S. and R. G. Palmer. 1987. Genetic analyses of non-fluorescent root mutants induced by mutagenesis in soybean. Crop Sci. 27:62-65.

Weber, C. R. 1967. Registration of Hark soybeans. Crop Sci. 7:403.

Reid G. Palmer - USDA ARS

IOWA STATE UNIVERSITY
Department of Zoology/Genetics &
Department of Agronomy
Ames, Iowa 50011
and
CLEMSON UNIVERSITY
Department of Agronomy and Soils
Clemson, South Carolina

Aneuploids from a male-sterile mutant from tissue culture

Aneuploids in soybean have been suspected to occur among progeny from tetraploid (2n=4x=80 chromosomes) plants (Sen and Vidyabhusan, 1960; Tang and Lin, 1963; Hu, 1968; Palmer, unpublished). Triploids have been excellent sources of aneuploids in many plants, but in soybean the results have not been encouraging (Chen, Heer, and Palmer, 1985; Chen and Palmer, 1985). Stelly et al. (1979) reported aneuploids from naturally occurring sterile plants in a commercial soybean field.The best source of aneuploids in soybean has been among the progeny of meiotic mutants (Palmer, 1974; Palmer and Heer, 1976; Palmer and Kaul, 1983; Skorupska and Palmer, 1987). Aneuploids have been found among progeny of the male-sterile female-fertile (ms) mutants (Sorrells and Bingham, 1979; Crane et al., 1982; Sadanaga and Grindeland, 1981; Zhang and Palmer, 1990). Addition aneuploids and deficient aneuploids were found among seed harvested from neutron-irradiated plants (Sadanaga and Grindeland, 1979).

We obtained seed of two sterility mutants derived from soybean cotyledonary node cultures (Graybosch et al., 1987). We have described the genetics of the 'Calland' sterile (Palmer et al, 1989). In this newsletter article we report the chromosome number and fertility/sterility of seed harvested from the 'Funman' sterile. Chromosome number was determined from root tip preparations (Palmer and Heer, 1973). All the aneuploid seedlings and six 40-chromosome plants were transplanted to the field. Pollen fertility was determined by I_2KI.

We obtained 10 aneuploid plants from 114 seedlings that were counted, (Table 1). Three 41-chromosome plants were fertile and produced seed. These aneuploid plants will be compared with the thirteen primary trisomics maintained in the soybean cytogenetic collection (Ahmad and Hymowitz, 1994).

Table 1: Chromosome number and fertility/sterility of plants grown from seed harvested on sterile plants from the tissue - culture derived 'Funman' sterile

Chromosome number	Number of plants		
	Fertile	Sterile	Not determined
40	5	1	98
41	3*	5	0
42	0	2	0

*One plant had some pollen sterility as determined by I_2KI staining.

References:

Ahmad, F. and T. Hymowitz. 1994. Identification of five new primary trisomics in soybean based on pachytene chromosome analysis. Genome 37:(in press).

Chen, L. F., H. E. Heer, and R. G. Palmer. 1985. The frequency of polyembryonic seedlings and polyploids from ms1 soybean. Theor. Appl. Genet. 69:271-277.

Chen, L. F. and R. G. Palmer. 1985. Cytological studies of triploids and their progeny from male-sterile ms1 soybean. Theor. Appl. Genet. 71:400-407.

Crane, C. F., W. D. Beversdorf, and E. T. Bingham. 1982. Chromosome pairing and associations at meiosis in haploid soybean (Glycine max). Can. J. Genet. Cytol. 24:293-300.

Graybosch, R. A., M. E. Edge, and X. Delannay. 1987. Somaclonal variation in soybean plants regenerated from the cotyledonary node tissue culture system. Crop Sci. 27:803-806.

Hu, S. 1968. Production, identification and characterization of heteroploids in Glycine max. (L.) Merrill. M.S. thesis. University of Illinois, Urbana.

Palmer, R. G. 1974. Aneuploids in the soybean, Glycine max. Can. J. Genet. Cytol. 16:441-447.

Palmer, R. G. and H. Heer. 1973. A root tip squash technique for soybean chromosomes. Crop Sci. 13:389-391.

Palmer, R. G. and H. Heer. 1976. Aneuploids from a desynaptic mutant in soybeans [Glycine max. (L.) Merr.]. Cytologia 41:417-427.

Palmer, R. G. and M. L. H. Kaul. 1983. Genetics, cytology, and linkage studies of a desynaptic soybean mutant. J. Hered. 74:260-264.

Palmer, R. G., H. S. Skorupska, and R. A. Graybosch. 1989. Allelism tests of a tissue-culture derived male-sterile, female sterile soybean mutant. Soybean Genet. Newsl. 16:109-111.

Sadanaga, K. and R. Grindeland. 1979. Aneuploids and chromosome aberrations from irradiated soybeans. Soybean Genet. Newsl. 6:43-45.

Sadanaga, K. and R. Grindeland. 1981. Natural cross-pollination in diploid and autotetraploid soybeans. Crop Sci. 21:503-506.

Sen, N. K. and R. V. Vidyabhusan. 1960. Tetraploid soybeans. Euphytica 9:317-322.

Skorupska, H. and R. G. Palmer. 1987. Monosomics from synaptic ks mutant. Soybean Genet. Newsl. 174-178.

Sorrells, M. E. and E. T. Bingham. 1979. Reproductive behavior of soybean haploids carrying the ms1 allele. Can. J. Genet. Cytol. 21:449-455.

Stelly, D. M., P. S. Muir, and R. G. Palmer. 1979. Spontaneously occurring sterile plants. Soybean Genet. Newsl. 6:45-47.

Tang, W. T. and C. C. Lin. 1963. Artificial induction and practical value of tetraploid soybeans. Bot. Bull. Acad. Sin. 4:103-110.

Zhang, F. and R. G. Palmer. 1990. Linkage studies with an unknown trisomic that occurred spontaneously in ms1 ms1 soybean plants. Soybean Genet. Newsl. 17:93-95.

R.G. Palmer - USDA
H. T. Skorupska

IOWA STATE UNIVERSITY
USDA/ARS Field Crops Research and Department of Agronomy
Ames, IA 50011

Physical mapping of polymorphisms linked to the *Phytophthora sojae* resistance locus *Rps*1

Introduction: The *Phytophthora sojae* resistance locus, *Rps*1, is commonly used in breeding programs to develop disease resistant soybean cultivars. *Rps*1 is unusually polymorphic encoding at least five, and potentially six, different alleles. Restriction fragment length polymorphism (RFLP) mapping has located *Rps*1 to linkage group N of the USDA-ISU soybean molecular genome map, approximately 2 cM from the RFLP locus A071-1 (Diers et al., 1992). We have shown that the A071-1 locus is also unusually polymorphic and that polymorphisms at this locus distinguish five groups of *Rps*1-containing germplasms (Polzin et al. 1994). This study examines the feasability of devising an allele-specific PCR assay of A071-1 polymorphism for marker assisted breeding of *Rps*1-containing cultivars.

Materials and Methods: The seven germplasms examined were Williams (*rps*1) (Moots et al., 1983), Mukden (*Rps*1-a) (Bernard et al., 1957), Sanga (*Rps*1-b) (Lam-Sanchez et al., 1968), Arksoy (*Rps*1-c) (Mueller et al., 1978), PI103091 (*Rps*1-d) (Buzzell and Anderson, 1992), PI172902 (*Rps*1-?) (T. Kilen, pers. comm.), and Kingwa (*Rps*1-k) (Bernard and Cremeens, 1981). DNA was isolated from the leaves of field-grown plants by the method of Kiem et al. (1988). Probe labelling and Southern hybridization was as described previously. All washes were at high stringency (0.1X SSC, 0.1% SDS, 65°C).

A clone containing the A071-1 locus was isolated from an EMBL3 genomic library of Williams 82 DNA (Clontech, Palo Alto, CA) as described previously (Polzin et al. in press). The entire A071-1 clone was divided into contiguous subfragments of 0.4 to 1.7-kb identified by restriction mapping. The subfragments were purified twice by electrophoresis through low melting point agarose (SeaPlaque, FMC, Rockland, ME) prior to being arrayed on dot blots or labelling for use as probes in Southern hybridizations.

Results and Discussion: RFLP analysis of Williams (*rps*1), Mukden (*Rps*1-a), Sanga (*Rps*1-b), Arksoy (*Rps*1-c), PI103091 (*Rps*1-d), PI172902 (*Rps*1-?), and Kingwa (*Rps*1-k) revealed that six of eight enzymes each uncovered three polymorphisms at A071-1. The A071-1 polymorphisms distinguished these germplasms into four groups: (Williams, Sanga, PI172092), (Mukden), (Arksoy, Kingwa), and (PI103091)

(Polzin et al. in press). To determine if the distinguishing polymorphisms might have occurred in low copy sequence suitable for conversion into PCR amplifiable polymorphisms the distribution of low copy and repetitive DNA surrounding the A071-1 locus was determined. An EMBL3 clone containing the A071-1 locus from Williams 82 was obtained as previously described (Polzin et al. in press). Williams 82 is an isogenic line of Williams containing the *Rps*1-k and A071-1 locus from Kingwa (Moots et al., 1983; Polzin et al., 1994). The clone was restriction mapped and the entire clone divided into contiguous subfragments of 0.4 -1.7 kb (indicated by the vertical marks beneath the map in Fig. 1). Subfragments were screened for highly repetitive sequence by hybridization to labelled soybean genomic DNA. No subfragment hybridized to labelled soybean genomic DNA indicating that the entire 14 kb surrounding the A071-1 locus was devoid of highly repetitive sequence. However, probing of five digests of soybean genomic DNA with the labelled subfragments indicated a 1.8 kb region contained low to middle repetitive sequence (>10 bands detected) (Fig. 1). The remainder of the clone consisted primarily of low copy sequence (2-10 bands detected) including two regions that appeared to be single copy sequence (Fig. 1). Thus, the majority of the DNA surrounding the A071-1 locus appeared to be low or single copy sequence potentially suitable for PCR amplification.

To determine the location of the polymorphisms detected by RFLP analysis restriction maps of the A071-1 region present in each of the *Rps*1-containing germplasms was prepared by Southern hybridization analysis. Genomic DNA from the *Rps*1-containing lines was digested with *Bgl*II, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, and *Taq*I and probed with each of the A071-1 low and single copy subfragments. The germplasms were scored for polymorphism at A071-1 compared to Kingwa. If the germplasm was not polymorphic for a band it was concluded to have the same restriction map as Kingwa in that region. However, if the Kingwa band was missing and a new band was present, the new fragment was concluded to have replaced the Kingwa band in that germplasm. In cases where a probe detected several fragments the Kingwa fragment corresponding to the A071-1 locus was identified by comparison to the restriction map of the A071-1 clone.

The results of the mapping experiment are presented in Fig. 2. No new polymorphisms were observed that distinguished between members of the groups determined previously by polymorphisms detected by pA071. Thus, members of the same group appear to be highly homologous in this region. All germplasms gave hybridizations patterns consistent with the A071-1 regions of the different germplasms being co-linear with that of Kingwa. Thus, no large (>1 kb) rearrangements, deletions

or insertions were detected. However, three of the four groups (Mukden; Arksoy and Kingwa; PI103091) contained unique restriction sites not found at the A071-1 locus of the other groups (indicated by the asterisk in Fig. 2). The two most closely related A071-1 loci were (Williams, Sanga, PI172902) and (Mukden), with the Mukden locus differing only by the presence of an additional *Hae*III site. The (Arksoy, Kingwa) group contained three unique sites, one of which was located in a region of repetitive DNA. The PI103091 A071-1 region was the most divergent containing five unique restriction sites relative to the other groups, all of which occurred in low copy sequence. Thus, all germplasms contained unique restriction sites within low copy sequence which might be amplified by PCR. The co-linearity of the maps as well as the fact that several fragments overlapping the polymorphic restriction sites were not polymorphic suggests that these unique restriction sites may be the result of independent point mutations. Interestingly, all but two of the polymorphic sites are located just within or immediately to the right of the A071-1 sequence in Fig. 2. These results suggest that this region may be undergoing a somewhat higher rate of mutation and that DNA sequencing of this region provides the best chance of uncovering additional distinguishing polymorphisms which might be useful in developing an allele-specific PCR assay for marker-assisted breeding of *Rps*1-encoded *Phytophthora* resistance.

Conclusions: The restriction maps of the region surrounding locus A071-1 in germplasms containing different *Rps*1 alleles suggests that a highly polymorphic region lies immediately adjacent to one side of the A071-1 locus. All but one of the polymorphisms lies in low copy DNA and all of the polymorphisms appear to be the result of point mutations, although small rearrangements (<1 kb) cannot be ruled out. These results indicate that it should be possible to develop allele-specific PCR assays for marker-assisted breeding of cultivars containing *Rps*1 by targeting the polymorphic portion of the A071-1 locus.

References:

Bernard, R.L. and C.R. Cremeens. 1981. An allele at the *rps*$_1$ locus from 'Kingwa". Soybean Genet. Newsl. 8:40-42.

Bernard, R.L., P.E. Smith, M.J. Kaufmann, and A.F. Schmitthenner. 1957. Inheritance of resistance to *Phytophthora* root and stem rot in the soybean. Agron. J. 49:391.

Buzzell, R.J. and T.R. Anderson. 1992. Inheritance and race reaction of a new soybean *Rps*1 allele. Plant Dis. 76:600-601.

Diers, B.W., P. Keim, and R.C. Shoemaker. 1992. Mapping of the *Phytophthora* resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. 32:377-383.

Keim, P., T.C. Olson, and R.C. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsl. 15:150-152.

Lam-Sanchez, A., A.H. Probst, F.A. Laviolette, J.F. Schafer, and K.L. Athow. 1968. Sources and inheritance of resistance to *Phytophthora*. Crop Sci. 8:329-330.

Moots, C.K., C.D. Nickell, L.E. Gray, and S.M. Lim. 1983. Reaction of soybean cultivars to 14 races of *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 67:764-767.

Mueller E.H., K.L. Athow, and F.A. Laviolette. 1978. Inheritance of resistance to four physiologic races of *Phytophthora megasperma* var. *sojae*. Phytopathology 68:1318-1322.

Polzin, K.M., L.L. Lorenzen, T.C. Olson, and R.C. Shoemaker. 1994. An unusually polymorphic locus useful for tagging *Rps*1 resistance alleles in soybean. Theor. Appl. Genet. in press.

Kayla M. Polzin
Dept. of Agronomy

Randy C. Shoemaker
USDA/ARS Field Crops Research

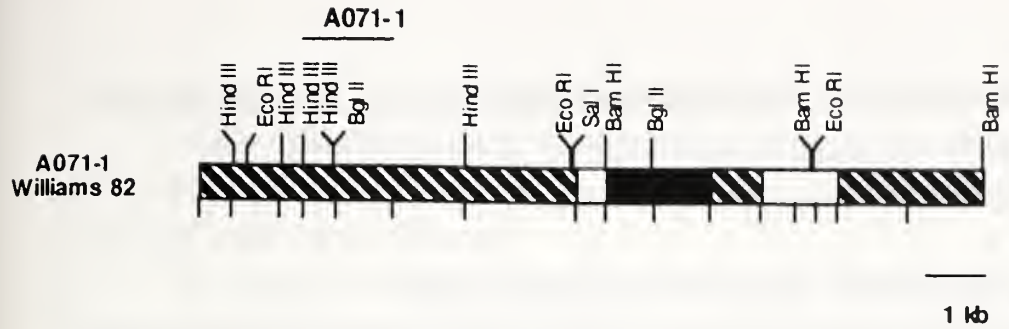


Fig. 1 **Distribution of low copy and repetitive sequence surrounding the A071-1 locus.** Hash marks beneath the map indicate the contiguous subfragments which were assayed for repetitive sequence. (□) = single copy sequence; (▨) = low copy sequence; (■) = repetitive sequence.

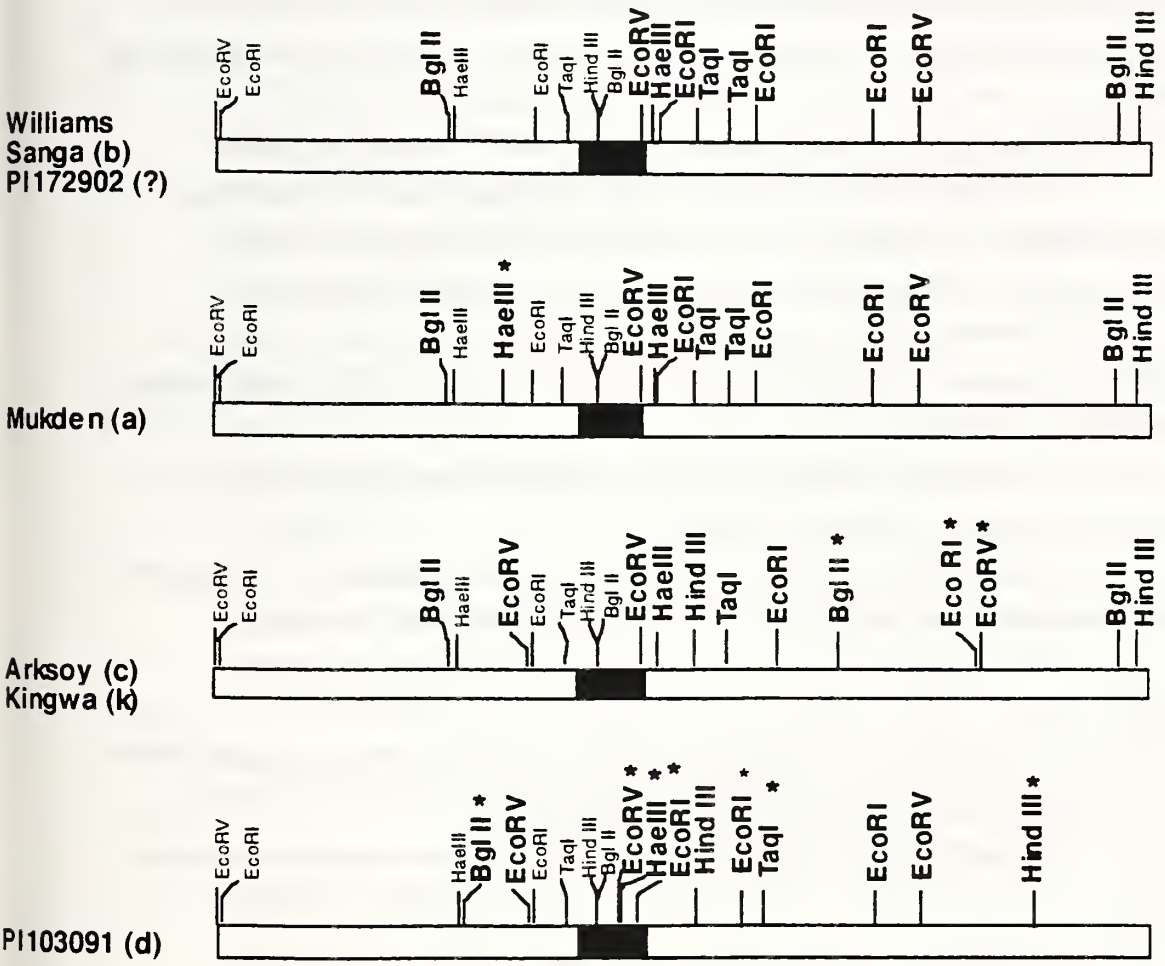


Fig. 2 **Restriction maps of the A071-1 regions in germplasms containing different Rps1 alleles.** The A071-1 locus is indicated by the black box. Restriction sites conserved in all germplasms are in plain type. Restriction sites present in only some of the germplasms are indicated by bold type. Restriction sites which are unique to one group are indicated by bold type and an asterisk.

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
Dept. of Agronomy
Urbana, IL 61801-4798

1)Cytological standards for the wild perennial *Glycine*, 1993

Table 1 contains a list of the wild perennial *Glycine* species, the three letter code, somatic chromosome number, and PI number of accessions used as cytological standards. The important points are as follows:

1. For genomic studies a standard set of cytological standards must be utilized.

2. If a standard for a species is crossed to another accession of the same species and perfect pairing is observed, then the second accession can be used as a standard.

3. The following changes have been made within the past two years:

a) The cytological standard for G. canescens was changed from PI 440928 to PI 440932. Australian workers requested the change based upon geographical origin of the accession and not upon cytological differences.

b) Previously, G. hirticaulis was reported as 2n=80 (Tindale and Craven, 1988). However, in our lab, the root tips of IL 1246 (=G 2876) had 2n=40 chromosomes.

c) Polyploid G. tabacina and G. tomentella are species complexes. See references below for accessions utilized.

d) Glycine tomentella at the diploid level is composed of at least three groups and is the subject matter for a Ph.D. dissertation by Jerry Hill.

References:

Kollipara, K.P., R.J. Singh and T. Hymowitz. 1993. Genomic diversity in aneudiploid (2n=38) and diploid (2n=40) Glycine tomentella revealed by cytogenetic and biochemical methods. Genome 36:391-396.

Kollipara, K.P., R.J. Singh and T. Hymowitz. 1994. Genomic diversity and multiple origins of tetraploid (2n=78,80) Glycine tomentella. Hayata Genome. 37: (in press).

Singh, R.J., K.P. Kollipara, F. Ahmad and T. Hymowitz. 1992. Putative diploid ancestors of 80-chromosome Glycine tabacina. Genome 35:140-146.

Tindale, M.D. and L.A. Craven. 1988. Three new species of Glycine (Fabaceae, Phaseolae) from northwestern Australia with notes on amphicarpy in the genus. Australia Systematic Botany. 1:399-410.

R.J. Singh
T. Hymowitz

2)Biosystematics of the genus *Glycine*, 1993

Table 1 contains a list of the wild perennial Glycine species, three letter codes, 2n chromosome numbers, genome symbol, and distribution of each species. The main points are as follows:

1. A new species had been added to the list - Glycine pindanica. Preliminary investigations suggest that the species is closely related to G. hirticaulis.
2. Variations in the trypsin and chymotrypsin inhibitor electrophoretic banding patterns in seed are useful for biosystematic studies in the genus Glycine.
3. Glycine tomentella (2n=40) appears to consist of at least three genomic groups.
4. Polyploid G. tabacina is a species complex containing accessions with A and B genomes (no adventitious roots; genomic allopolyploid) or two B genomes (with adventitious roots; segmental allopolyploid).
5. Glycine tomentella (2n=78,80) are complexes. See reference below for detailed information.

References:

Kollipara, K.P. and T. Hymowitz. 1992. Characterization of trypsin and chymotrypsin inhibitors in the wild perennial Glycine species. J. Agric. Food Chem. 40:2356-2363.

Kollipara, K.P., R.J. Singh, and T. Hymowitz. 1992. Genomic diversity in aneudiploid (2n=38) and diploid (2n=40) Glycine tomentella revealed by cytogenetic and biochemical methods. Genome 36:391-196.

Singh, R.J., K.P. Kollipara, F. Ahmad, and T. Hymowitz. 1992. Putative diploid ancestors of 80-chromosome Glycine tabacina. Genome 35:140-146.

Tindale, M.D. and L.A. Craven. 1993. Glycine pindanica (Fabaceae, Phaseolae), a new species from West Kimberly, Western Australia. Australian Systematic Botany. 6:371-376.

R.J. Singh
T. Hymowitz

3) Management of the USDA wild perennial *Glycine* collection, 1993

Under a specific cooperative agreement between the University of Illinois and the USDA, the wild perennial Glycine collection is being maintained at the University of Illinois. Table 1 contains the names of the species, somatic chromosome numbers, and number of accessions. The major points are as follows:

1. Two shipments containing a total of 33 new accessions were received during 1993. All of the new accessions were collected in Australia. Thirty-six accession were collected by Brown, Grace (both from CSIRO/Canberra) and Hymowitz from Western Australia. CSIRO has sent 29 accessions from this trip, including seed of a new species, G. pindanica.
2. A number of accessions are difficult to grow through maturity and seed production. To counter this, these lines have been intensively propagated by means of cuttings and grafts onto G. max rootstocks. Seeds are surface sterilized and germinated in autoclaved media. This has allowed some accessions of G. tomentella to be multiplied for the first time.
3. In order to protect employees from dermal exposure to toxins, and in response to the development of multiple pesticide resistance in greenhouse pests, the perennial greenhouse has been has been converted to a biological control regime. The following measures have been adopted:

    a. Screening: all vents have been screened to prevent an influx of nuisance and pest insects

    b. Appropriate chemicals: Insecticidal soaps and horticultural oils are useful for spot treatments as they have no residual toxicity and no harmful fumes. A mixture of Sunspray oil and baking soda is a useful spray for powdery mildew.

    c. Predaceous and parasitic organisms:

        1. Mealybugs: Cryptolaemus beetles (predatory ladybird beetles) and Leptomastix wasps (parasites)
        2. Whiteflies: Encarsia (parasitic wasp)
        3. Thrips: Neoselius (predatory) mites
        4. Spider mites: Mesoselius longipes (low humidity tolerant) and Neoselius californicus (starvation resistant) predaceous mites.
        5. Aphids: lady bird beetle
        6. Fungus gnats: Gnatrol, a Bacillus thuringensis drench

Table 1. Wild, perennial Glycine species, somatic chromosome number, and number of accessions.

	Species	2n	Number of Accessions
1.	G. albicans	40	2*
2.	G. arenaria	40	3
3.	G. argyrea	40	3
4.	G. canescens	40	77
5.	G. clandestina	40	138
6.	G. curvata	40	5
7.	G. cyrtoloba	40	28
8.	G. falcata	40	16
9.	G. hirticaulis	40	2*
10.	G. lactovirens	40	2*
11.	G. latifolia	40	43
12.	G. latrobeana	40	12*
13.	G. microphylla	40	24
14.	G. pindanica	40	3
15.	G. tabacina	40	20
		80	132
		?	97
16.	G. tomentella	38	10
		40	30
		78	54
		80	53
		?	125

* Recalcitrant species with regard to seed multiplication

These organisms may be obtained from any of a number of companies, including the following:

IPM Laboratories, Inc.
P.O. Box 300
Locke, NY 13092-0300

Biotactics, Inc
7765 Lakeside Drive
Riverside, CA 92509

Results have been quite encouraging. The high initial costs of the control organisms have been offset by the resistance of the greenhouse to reinfestation and a reduction in chemical expenses. With increasing pesticide regulation and longer reentry restrictions, reducing pesticide levels makes the greenhouse more useful to researchers. As no biological controls exist for fungus diseases, the plants are drenched with Banrot, a broad-spectrum fungicide, about every six weeks. As required by law, Jean Burridge is a licensed Public Applicator, Demonstration and Research category.

4. During the year, 27 seed requests for wild perennial material were received. A total of 366 packets of seed were shipped. A standard packet has five seeds per accession. Domestically, seed was shipped to Alabama, Illinois, Iowa, Minnesota, Puerto Rico and Utah. Internationally, seed was shipped to Canada, India, Israel, Russia and the United Kingdom.
5. Voucher specimens of all accessions grown out in the greenhouse were placed in the Crop Evolution Herbarium (CEL).
6. The inventory of the collection is maintained on a Dell 450/L personal computer.
7. Sixty packets of 50 seed each were sent to the National Seed Storage Laboratory in Fort Collins, Colorado for long term storage. Thus far, a total of 596 accessions have been sent to this facility. Another emergency set, containing 10 seeds per packet, was sent to Dr. R.L. Nelson, Curator, USDA Soybean Germplasm Collection, Urbana, IL. PI numbers are requested when an accession has been successfully multiplied. Seeds of all accessions in the collection are stored in envelopes, in a milk cooler set to four degrees Fahrenheit.

8. To request seed, please write to:

Dr. T. Hymowitz
Department of Agronomy
University of Illinois
AE-110 Turner Hall
1102 S. Goodwin Ave.
Urbana, Illinois 61801
USA

J.A. Burridge
T. Hymowitz

4) Root fluorescence in the Genus *Glycine* subgenus *Glycine*: Revisited

Parot (1982) screened 172 accessions representing seven wild perennial *Glycine* species for diversity of root fluorescence. The objective of the research reported herein was to determine the diversity of root fluorescence in 473 accessions representing twelve wild perennial species in the subgenus Glycine.

To screen for root fluorescence, five seeds of each accession were scarified, placed on damp filter paper in a 100x15mm petri dish and then put in the dark for four or five days. The roots were observed under a 365 mm UV light source. Observations of fluorescence were made on: the growing point, the main portion of the root, the entire and or top portion of the hypocotyl, and the root's intensity of fluorescence. However, the seedlings were classified as being fluorescent, nonfluorescent, or unusual. The accession PI 399478 Glycine canescens was used as the standard nonfluorescent type, while accession PI 233138 Glycine clandestina was used as the standard fluorescent type.

Of the 473 accessions tested, G. canescens, G. clandestina and G. tomentella were found to contain nonfluorescent genotypes (Table 1). The accessions that do not have root fluorescence are listed in Table 2. Within G. curvata, three accessions had an unusual fluorescent hue (Table 1). This was reported previously by Parot (1982).

References:

Parot, C. 1982. Root Fluorescence in the genus Glycine subgenus Glycine. Soybean Genet. Newsl. 9:115-117.

Charletta Little
Theodore Hymowitz

DEPARTMENT OF CROP AND SOIL SCIENCES
University of Georgia
Athens, Georgia 30602-7272 USA

Soybean DNA isolation procedure using fresh tissue

Fresh soybean tissue may be used in a DNA isolation procedure that employs modifications of Doyle and Doyle (1990), coupled with a slightly modified Rowland and Nguyen (1993) PEG (polyethylene glycol) purification procedure. This method is rapid, amenable to fresh tissue, and yields DNA that is purified for use in restriction enzyme digests and PCR. Freeze drying, liquid nitrogen, RNA removal and cesium chloride gradients are not necessary. This procedure may be used for fresh, frozen or freeze dried tissue.

Procedure: Grind up to one gram fresh somatic embryos or leaf tissue (youngest are best) in 20 ml 70°C CTAB buffer [2% w/v CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 2% w/v PVP-40 (polyvinylpyrrolidone) Sigma Chemical Co., St. Louis, MO], 5 mM ascorbic acid, 4.0 mM DIECA (diethyldithiocarbamic acid) Sigma Chemical Co., St. Louis, MO], using a mortar and pestle. Just prior to homogenization, add 60 µl of 2-mercaptoethanol to mortar. Immediately following homogenization, transfer the homogenate to a 50 ml conical tube and incubate at 70°C for five minutes. Extract the homogenate with 20 ml chloroform-isoamyl alcohol (24:1 v/v). Shake tubes horizontally for five minutes at 500 rpm (IKA- Vibrax - VXR, Cincinnati, OH) and centrifuge (1000 x g at 22°C) in a clinical centrifuge for five minutes to separate phases. Transfer the upper, aqueous, DNA-containing phase to a fresh tube using an upside-down 25 ml serological pipette. Centrifuge (1000 x g at 22°C) for five minutes. Cellular debris and contaminants will pellet. Transfer the DNA-containing supernatant to an Oak Ridge tube. Add 4 ml 0.7 M NaCl, 5% CTAB (Rowland and Nguyen, 1993) and precipitate DNA with 0.7 volume isopropanol for ten minutes at 4°C and centrifuge (8000 x g) for 20 minutes. Air-dry the pellet and resuspend the DNA in 500 µl of TE, and transfer to a 1.5ml microfuge tube. An additional precipitation using PEG increases restrictability (Rowland and Nguyen, 1993). Precipitate the DNA by adding 125 µl 4M NaCl and 625 µl 13% PEG (Rowland and Nguyen, 1993). After one hour incubation at 4° C centrifuge at 14,000 rpm in a microfuge for ten minutes at 22°C. Rinse the pellet once with 70% ethanol and centrifuge as above. Resuspend the DNA in 500 µl TE. This should provide 0.3 to 0.5 mg DNA. No RNA removal treatment is presented since RNA has not been observed (in my hands) to interfere with restriction enzyme digests. DNA may be

quantified using fluorometry or spotting on an agarose gel with ethidium bromide staining.

References:

Doyle, J.J., and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Rowland, L.J. and B. Nguyen. 1993. Use of polyethylene glycol for purification of DNA from leaf tissue of woody plants. BioTechniques 14:735-736.

C. Neal Stewart, Jr.

UNIVERSITY OF UTAH
Department of Biology
Salt Lake City, UT 84112

Molecular phylogeny as a tool for soybean breeding III

Lark et al. 1992; 1993 used RAPIDLY AMPLIFIED POLYMORPHIC DNA (RAPD) to establish soybean phylogeny relative to perennial and annual species. We report here, as a third installment in this series, the results collected in an undergraduate laboratory (Biology 360) at the University of Utah in the Fall of 1993. In this set, we included DNA from several different genera outside the *Glycine* genus as well as some additional *G. soja* and *G. max* to further elucidate specific relationships. Key findings in this year's results include: 1) a close relationship between the *Glycine* subgenus *Glycine* (i.e., the perennials) and *Neonotonia* (formerly included in the *Glycine* genus); 2) further congruence with independently published phylogenies of *Glycine* ;
3) some interesting biogeographical patterns in the *G. soja* accessions; 4) a clarification of G. *clandestina* 440.948 relationships to similar species found by Lark et al. 1993; and 5) contrasting patterns of relationships between *G. gracilis* accessions and other *Glycine* species.

Materials and Methods

The origin of seeds we used have previously been described by Lark et al. 1992; 1993. Seeds of taxa not previously described were obtained from T. Hymowitz at the University of Illinois.

DNA was isolated and RAPD markers were determined using similar procedures to those described by Lark et al., 1992; 1993. In summary, isolated genomic DNA was used as a template in PCR reactions with 29 different 10mer primers, and the products were separated on agarose gels. Ethidium bromide visualized bands on gels were captured onto a MACIIci computer through a TV camera using the NIH Image 1.42 program. Images were enhanced to clarify banding patterns and then scored relative to standard Kb ladders. The characters were specific size DNA fragments resulting from a specific primer PCR. Binary character states were assigned: taxa were labeled as either having ('one') or not having ('zero') a particular-sized fragment for each primer. Two sets of taxa were used with each primer and each set was run and scored on separate gels.

Scores from both gels of the same primer were not combined because the 20-35 different sized fragments on each gel were difficult to align accurately across both gels. Therefore, we analyzed each set of taxa separately. Included in this year's taxa were four *G. max*, nine *G. soja*, one *G. gracilis*, nine perennial *Glycine* species, and one non-*Glycine* on gel one, and nine *G. max*, one *G. gracilis*, three *G. soja*, seven *Glycine* perennials, Chinese black, and four non-*Glycine* genera on gel two.

We used an heuristic approach within Phylogenetic Analysis Using Parsimony (PAUP 3.1.1) (Swofford 1993) to produce phylograms. When less than ten taxa were analyzed, exact search methods were used for finding trees.

Results and Discussion

Evaluation of Neonotonia wightii relative to Glycine and non- Glycine species

Neonotonia wightii (Wight & Arn.) Lackey, a one species genus, is a pasture legume (Rhodesian kudzu) found primarily in Africa and rarely in Asia (Lackey 1981). Early classification schemes of the Tribe Phaseoleae included *Neonotonia* in the genus *Glycine* (formerly, *G. wightii*) based on morphology (see review by Hymowitz and Singh 1987). Lackey in 1977 removed *G. wightii* from the *Glycine* genus altogether based on morphological, chromosome and biochemical features and on a geographic distribution that contrast with all other *Glycine* species (Hymowitz and Singh 1987).

Within the Glycininae subtribe, the genus *Sinodolichos* is proposed as being more allied with the genus *Glycine* , while another set of Glycininae genera, including *Neonotonia* and *Amphicarpa* , are considered as derived along a separate path (Lackey 1981).

We were interested in examining the relationships of these non- *Glycine* species (especially *Neonotonia*) to the *G. soja* and *G. max* accessions. Initially, we used *Cajanus cajan* (L.) Millsp. (pigeon pea) (subtribe Cajaninae) as the outgroup because it was the only taxa not in the subtribe Glycininae. We tested the robustness of phylograms by including/excluding various taxa.

In the phylogram produced with all taxa included (Fig. 1a), *Amphicarpa bracteata* and *Sinodolichos* is closely aligned with *Cajanus,* with *Sinodolichos*

being the root to the *Glycine* genus. The *Glycine* genus forms two subclades divided between the *Soja* (i.e., *G. max* and *G. soja*) and *Glycine* (i.e., perennial species) subgenera. This phylogeny agrees with the proposed relationships of Lackey (1981) except for *Neonotonia*. This non-*Glycine* genus links as a sister taxon to *G. argyrea* and is distant from the other non-*Glycine* genera.

Adjusted character and patristic distances between *Neonotonia* and all other taxa produced in phylogram 1 were examined (Table 1). Character distances represent absolute differences in character states between taxa. Patristic distances are derived values based on the phylogeny inferred from any particular set of assumptions (e.g., parsimony in our analyses). The ratio of patristic to character distance, also a derived measure, is also included in the table. The ratio provides a quick way to compare the increase in number of homoplasies (i.e., convergences and reversals in character states) relative to the character distance between taxa that result from the most parsimonious phylogram. In terms of character distances, *Neonotonia* is much closer to the *Glycine* taxa, particularly *G. argyrea*, than it is to non-*Glycine* taxa (Table 1). Moreover, *G. argyrea* and *Neonotonia* share 70% of their character states in common, which is among the highest percent we observed between any pair of perennial taxa (complete data not shown). This closeness between *Neonotonia* and the perennial *Glycine* species is also observed in the patristic distances and ratios (Table 1), but the closeness between *Neonotonia* and the *G. max* is not observed patristically.

Fig. 1

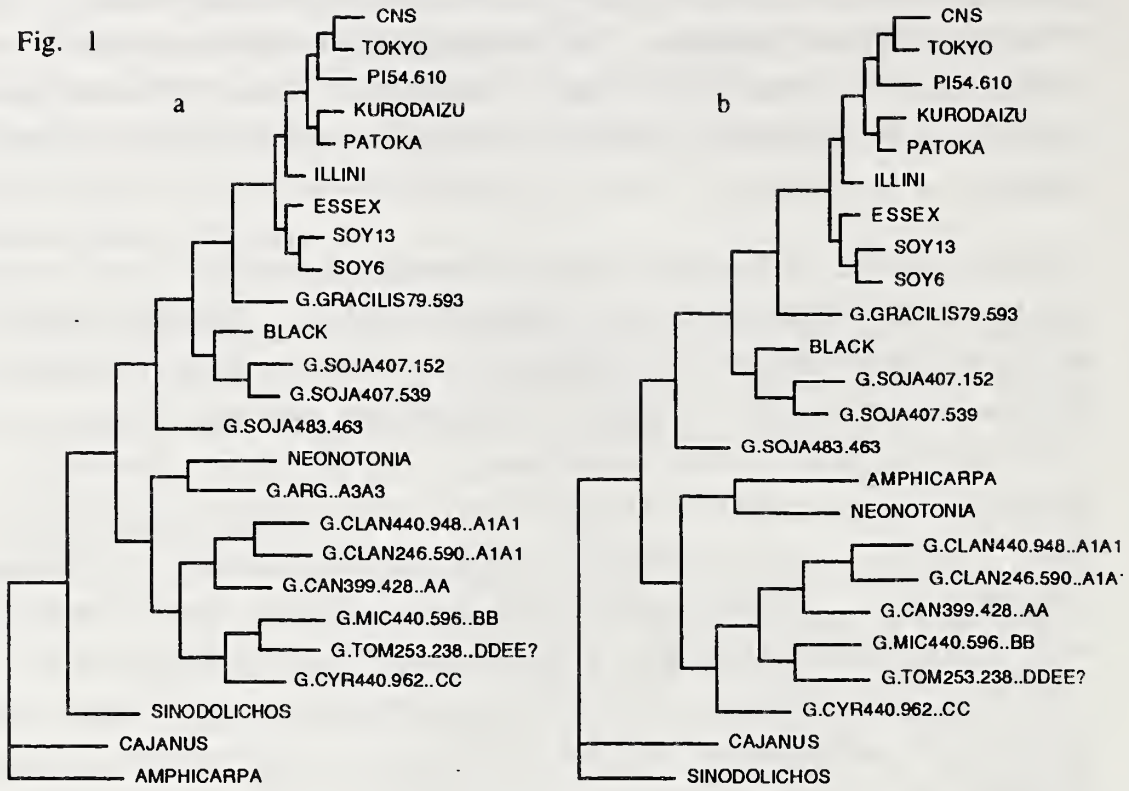


Table 1. The character and patristic distances and ratio of patristic to character distances between *Neonotonia wightii* and each taxa on gel two. Taxa are ranked by their character distance from *Neonotonia*. Data are from results of phylogram 1 (Fig.1).

Plant species			*Neonotonia* distance		
Lane		Accession	character	patristic	ratio
14	*G. argyrea*	PI 505.151	164	164	1
16	*G. clandestina*	PI 246.590	204	304	1.49
21	Chinese black		208	316	1.52
2	*G. max*-Tokyo	PI 8.424	209	414	1.98
7	*G. max*-Essex		209	370	1.77
6	*G. max*-Illini		213	358	1.68
5	*G. max*-Patoka	PI 70.218	216	471	2.18
8	Soy#13		217	394	1.82
13	*G. gracilis*	PI 79.593	217	352	1.62
3	*G. max*-	PI 54.610	218	474	2.17
9	Soy#6		218	384	1.76
15	*G. clandestina*	PI 440.948	219	301	1.37
23	*G. soja*	PI 407.152	219	359	1.64
19	*G. cyrtoloba*	PI 440.962	220	276	1.25
20	*G. tomentella*	PI 253.238	221	315	1.43
24	*G. soja*	PI 407.539	223	343	1.54
18	*G. canescens*	PI 399.428	224	260	1.16
4	*G. max*-Kurodaizu	PI 81.041	225	474	2.11
22	*G. soja*	PI 483.463	225	276	1.23
1	*G. max*-CNS	PI 71.597	227	450	1.98
25	*Sinodolichos* black		228	298	1.31
17	*G. microphylla*	PI 440.596	235	321	1.37
10	*Cajanus cajun*	CU188	241	389	1.61
11	*Amphicarpa bracteata*	CU169	248	404	1.63

With *G. argyrea* removed from the analysis, *Amphicarpa* replaces *G. argyrea*'s position as a sister taxon to *Neonotonia* in the perennial subclade (Fig. 1b). Our molecular data support the closeness of *Neonotonia* to the *Glycine* taxa but indicate that the most simplistic relationship (Fig. 1a) is dependent upon *G. argyrea* whose removal produces a phylogram reminiscent of Lackey's subdivision, in which *Neonotonia* and *Amphicarpa* are more closely aligned (Lackey 1981).

The results here thus question whether *Neonotonia* should have been removed from the *Glycine* genus. The data suggest that these two taxa are more closely related by ancestry than indicated by phenetic relationships. Certainly, *Neonotonia* appears to be at least as closely related to *Glycine* taxa as is *Sinodolichos* (Fig. 1 and also by character distance, data not shown), and *Neonotonia* is closer to the *Glycine* than it is to other non-*Glycine* genera. However, certain *Neonotonia* characteristics that contrast with *Glycine* are hard to resolve, particularly, the larger-sized and different base number of chromosomes of *Neonotonia* (2n= 22 or 2n=44) compared with other *Glycine* (2n=40 of most *Glycine* species). Biochemically, *Pueraria,* the proposed more ancestral genus of Glycininae, and *Neonotonia* are the primary genera that have canavanine, and none of the *Glycine* species contain this secondary plant compound (Lackey 1981). Further, the closeness of the genomes of *Neonotonia* and *G. argyrea* is troublesome based on geographic differences, the former being primarily distributed in eastern Africa and the latter found in a limited band of forest in eastern Australia (Grant et al. 1986). Perhaps these two species have arisen from a common ancestor that was present in Gondwana or Pangea prior to the separation of the continents.

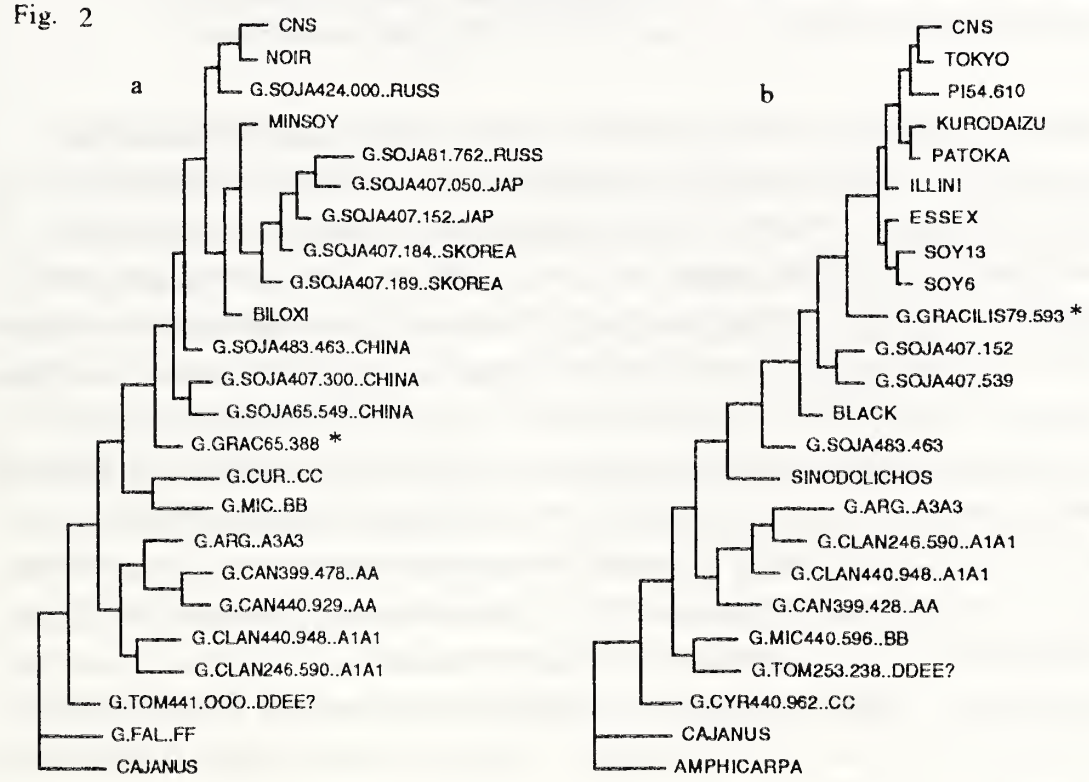
Relationships within the Glycine genus (subgenera Glycine and Soja)

Lark et al. 1993 found that species of the perennial subgenus *Glycine* grouped into separate clades consistent with genomic groupings developed by Doyle et al. 1990 and Hymowitz and Singh 1992. The results of this year's analysis (which includes more accessions and species) in both set of taxa agree with this pattern (Fig. 2a, b), further confirming the taxonomic grouping. All the species with AA genomes (*G. argyrea*, *G. canescens*, *G. clandestina*) form a subclade separate from the FF (*G. falcata*), CC (*G. curvata*), BB (*G.* microphylla), and DD or EE (*G. tomentella*) genomes.

Previously, Lark et al. 1992 indicated a clear geographic clustering of various *G. soja* accesssions. This year's data set includes a broader array of *G. soja* accessions in terms of geographic spread as well as duplicates from each country including two variants from Russia, two from Japan, two from South Korea and three from China. A similar geographic pattern emerges with those

from China being closest to *Glycine* perennials in terms of character and patristic distances (Fig. 2a). The two new Russian accessions are, however, distant from each other. The Chinese *G. soja* and the Russian *G. soja* 424.000 are patristically close to the *G. max.* *G. soja* 424.000 divides the *G. max* between CNS/Noir and Minsoy/Biloxi and is closer to the Chinese *G. soja* than the remaining *G. soja*. Because the domestication of soybean first occurred in northern China, possibly near the Russian border, the relationships shown in the phylogram may reflect a biogeographical pattern that parallels the selection of the cultivated soybean.

Fig. 2



G. clandestina 440.948 relationship with other Glycine taxa

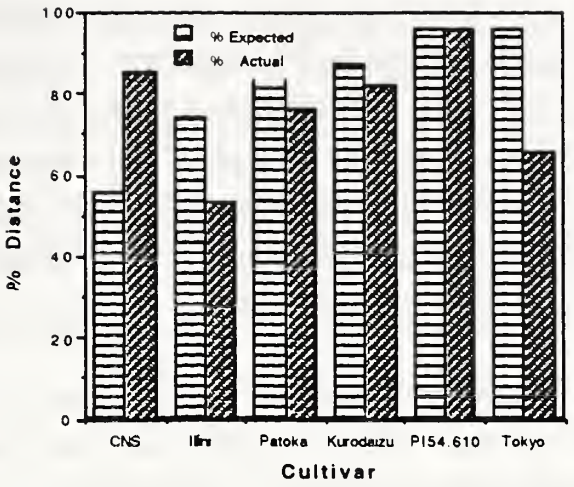
Lark et al. 1993 reported anomalous relationships between *G. clandestina* 440.948 and other perennials. *G. clandestina* 440.948 was not closely aligned with other accessions of the same species (*G. clandestina* 246.590) or with other *Glycine* species with similar A1A1 or AA genomes (*G. canescens* 399.428; 440.928) (see Lark et al. 1993, Fig. 1). Moreover, *G. clandestina* 440.948 was closer to the *G. max* cultivars than any other perennial. This year's data set, however, refute some of these relationships (see Fig. 2 a,b).

Both of these phylograms and the associated character distances agree with published phylogenies (Doyle et al. 1990; Hymowitz and Singh 1992) as shown above. The *G. clandestina* accessions form subclades which include other A1A1 or AA genomes (*G. canescens* and *G. argyrea*). Because this year's data agree with published data, we compared the *G. clandestina* and *canescens* lanes from seven 1992-93 (Lark et al. 1993) and 1993-94 gels (seven primers) to determine where differences in banding patterns occurred. It appeared that *G. canescens* 399.428 and *G. clandestina* 246.590 labels were interchanged in the earlier data set. When this was corrected, the alignment of taxa in the phylogram more closely matched the predicted pattern as in Fig. 2. However, the close relationship between the *G. max* cultivars and *G. clandestina* 440.948 (shown by Lark et al. 1993) was reproduced.

Evidence of selection among cultivated and natural soybean populations

1) Lark et al. 1993 showed that the actual character distance between the cultivar Essex and members of its pedigree did not agree with the expected values based on potential relatedness (i.e., 100- (% of the parental genome expected)). Tokyo, another taxon in the Essex pedigree, was added this year to further compare the actual with predicted values. As before, there was some agreement between the expected genomic and actual character distances, but the difference between actual and expected contributions of CNS to Essex was again observed. Because CNS has been used three times in different background crosses leading to Essex, the expected relatedness between the two genomes is high; however, CNS is very underrepresented in the Essex genome (Fig. 3). The selection process by breeders is a likely cause for this discrepancy. CNS is widely used in many crosses because it possesses the gene (rxp) that confers bacterial pustule resistance (Bernard et al. 1988). During the selection of the F1 generation of cross breeding, plants are more likely chosen for yield (and/or seed oil or protein) than for bacterial pustule resistance, which may or may not be present in a breeder's field. In addition, there could be a yield cost to plants that possess the resistance gene. If so, there would be further selection away from CNS.

Fig. 3



2) Following this example from domesticated soybeans, we also found that two *G. gracilis* accessions (wild introgressions) varied in their phylogenetic positions relative to the *Glycine* perennials and *Soja* subgenus (Fig. 2a,b). One hypothesis to explain the difference in relationships is that natural (rather than artificial selection) has played a role in separating the two accessions.

G. soja, *G. gracilis* and *G. max* are considered as the wild, the weedy and the domesticated soybean (Lackey 1981). *G. gracilis* is thought to be a hybrid between a *G. max* and *G. soja* and hybridization studies confirm this possibility (Singh and Hymowitz 1989). The three species are similar in some morphological traits, chromosome number (all have 2n=40), trypsin inhibitor banding patterns, geographical distribution and certain life history traits (all are annual inbreeders).

Two variants of *G. gracilis* (PI 65.388 and PI 79.593) were examined relative to their phylogenetic position between *G. max* and *G. soja*. Only one variant is present on each gel. While *G. gracilis* 79.593 forms a link between the *G. soja* and *G. max* accessions (as would be predicted if it were a hybrid between the two) (Fig. 2b), *G. gracilis* 65.388 forms a link between the *G. soja* accessions and perennial *Glycine* species (Fig. 2a). Several possibilities could account for this contrast in position. A) Each variant could have been the result of a different cross of quite distinct parents. PI 65.388 may have been

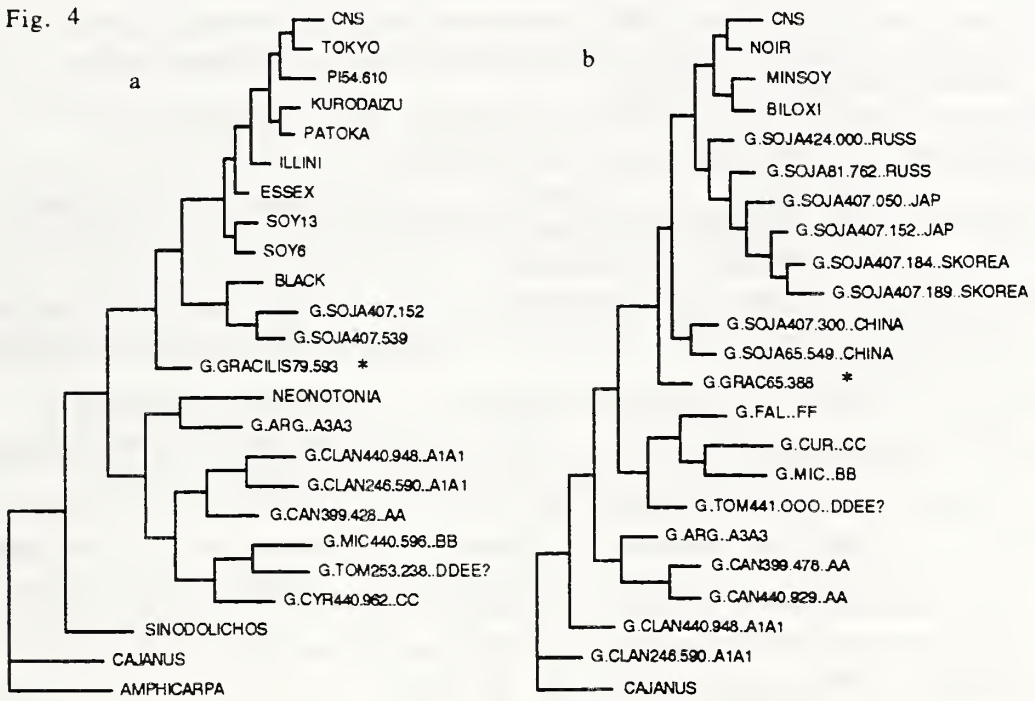
a cross between a more wild-like *G. max* and *G. soja* than the other *G. gracilis* accession. (Lark et al. 1993 demonstrated that some *G. max* are consistently closer patristically to the *Glycine* perennials than others (see Lark et al. 1993, Figs. 3, 4)). B) The relationship may be the result of selection or drift after hybridization. When *G. soja* and *G. max* hybridized and produced the heterozygous F1 progeny, certain lines may have lost chromosomes or parts of chromosomes differentially from one parent or the other during later generations of inbreeding as the plant line became more homozygous. This could give rise to contrasting genomic lines from the same set of parents.

C) Finally, the two variants may not be as different from the *G. max* and *G. soja* as the phylograms suggest, but the difference in positions may result from the specific taxa used in creating the phylogeny. To test this, we examined the average character distances between each variant and other *Glycine* taxa (Table 2). Both *G. gracilis* are much closer to the subgenus *Soja* than they are to the perennials. They both share similar proportions of character states in common with the *G. max* and with the *Glycine* perennials. However, they are different in their character distance from the *G. soja*. This difference to *G. soja* was found to be due to one taxon, *G. soja* 483.463 (Table 2). This was confirmed when phylograms were created lacking this taxon (Fig. 4a,b). As can be seen, the omission of *G. soja* 483.463 results in two very similar phylograms with respect to *G. gracilis* . Both form a link between between the *Soja* subgenus and the perennial *Glycine* species (Fig. 4 a, b) and are equidistant from *G. soja* and *G. max*. This contrast in genomic similarity to *G. soja* 483.463 could be the result of A) distinct sets of parents or B) natural selection/ non-random segregation as discussed above.

Table 2. The average character distance and % shared character states between each *G. gracilis* accession and other *Glycine* taxa. Data are from Fig. 2a,b phylogram.

Character measure	*G. gracilis* accession	*G. max*	Perennial *Glycine*	*G. soja*	*G. soja* 483.463
Character distance	79.593	136	202	167	182
	65.388	118	175	107	91
% shared character states	79.593	75.4	63.5	69.8	67.1
	65.388	75.1	63.1	77.4	80.8

Fig. 4



In summary, RAPD markers have been a useful tool in further elucidating the basic phylogeny of soybeans and related genera as well as potential patterns of selection (natural or artificial) of wild and domesticated soybeans. This technique, although it involves many technical steps that require great care to insure accuracy and repeatability among samples, can be accomplished in an undergraduate class laboratory. It has the advantage of being a non-radioactive technique that samples throughout the genome and may be useful in identifying potential breeding partners among allies or sister taxa.

Acknowledgement

This research was produced in an undergraduate laboratory, Molecular Evolution, Biology 360. This is part of an undergraduate initiative supported by a grant from the Howard Hughes Medical Institute to the Department of Biology, University of Utah.

References

Bernard, R. L., G. A. Juvik, E. E. Hartwig and C. J. Edwards. 1988. Origins and Pedigrees of Public Soybean Varieties in the United States and Canada. USDA Technical Bulletin #1746.

Doyle, J. J. , J. L. Doyle and A. H. D. Brown. 1990. A chloroplast -DNA phylogeny of the wild perennial relatives of soybean (*Glycine* subgenus *Glycine*): congruences with morphological and crossing groups. Evolution 44: 371-389.

Hymowitz, T. and R. J. Singh. 1987. Taxonomy and speciation. pp 23-48 in Soybeans: Improvement, production and uses. J. R. Wilcox (ed) American Agronomy Society.

Hymowitz, T. and R. J. Singh. 1992. Biosystems of the genus *Glycine*. Soybean Genetics Newsletter. 19: 174-181.

Grant, J. E., R. Pullen, A. H. D. Brown, J. P. Grace and P. M. Gressholf. 1986. Cytogenetic affinity between the new species G. argyrea and its congeners. J . Heredity 77: 423-426.

Lackey, J. A. 1981. Tribe 10. Phaseoleae. pp. 301-327 In Advances in Legume systematics. Vol 2. Polhill, R. M. & P. H. Raven (eds) Proc. International Legume Conf., Kew

Lark, K. G., J. Evans, F. Basha, R. Bogden, R. Copeland, R. Ellison, D. Horne, K. Lee, K. Mcdonald, J. Pierson, W. Schuster, P. Wilhelm and Y. Yu. 1992. Molecular phylogeny as a tool for soybean breeding. Soybean Genetics Newsletter 19: 174-181.

Lark, K. G., J. Evans, P. Wilhelm, S. Atkinson, M. Christensen, B. Crockett, B. L. Dunford, C. E. Jones, S. Mendenhall, T. E. Messick, C. C. Prout, P. E. Solomon, M. E. Stevens, I. Terry, C. Walker, C. L. Wong and R. Ellison. 1993. Molecular phylogeny as a tool for soybean breeding II. Soybean Genetics Newsletter 20: 197-201.

Singh, R. J. and T. Hymowitz. 1989. The genomic relationships among *Glycine soja* Sieb. and Zucc., *G. max* (L.) Merr. and '*G. gracilis*' Skorvtz. Plant Breeding 103: 171-173.

Swofford, D. M. 1993. Manual for PAUP 3.1.

L. I. Terry*
K. G. Lark*
L. Allphin**
M. Stevens**
A. J. Angilau***
M. Gay***
L. Harrison***
B. Huff***
P. Magasich***
M. Miller***
G. Nishimoto ***
C. Rhead***
Q. Sahratian***
J. Whitehead***
J. A. Whitehead***

*** professors**
**** teaching assistants**
***** students**

THE UNIVERSITY OF ILLINOIS
Department of Agronomy and USDA/ARS
1101 W. Peabody Dr.
Urbana, IL 61801

A Third Allele at the dt1 Locus

Introduction: The gene pair Dt1 dt1 was first reported by Woodworth (1933) based on F2 segregation in a cross between the indeterminate cv. Manchu (Dt1) from northeast China and the determinate cv. Ebony (dt1) from Korea. Similar segregation for this trait has been observed by many workers since then. Because of the major effect of this gene pair on stem termination soybean cultivars are commonly categorized as either "determinate" or "indeterminate". With determinate stem type (dt1 dt1) there is usually little growth in stem length after flowering whereas with indeterminate stem type (Dt1 Dt1) stem elongation and node production continues after flowering producing a longer more tapered stem. The heredity of this trait and the intermediate phenotype of the heterozygote were presented in more detail by Bernard (1972). There is considerable variation in stem growth within each of these two types, with time of flowering and time of maturity having major effects on stem morphology. In addition some distinctly intermediate types have been observed. Bernard (1972) studied the intermediate stem type of strain T117 from the Genetic Type Collection which he called semi-determinate and found it to be controlled by a dominant gene (Dt2) hypostatic to dt1 dt1 and quite similar in phenotype to the heterozygote Dt1 dt1.

Materials and Methods: To study the phenotype and breeding value of the two gene pairs (Dt1 dt1 and Dt2 dt2) we developed near-isogenic lines by backcrossing to the Group IV indeterminate cultivar Clark (Johnson 1958) and selecting from the BC5 (Bernard et. al. 1991). The initial dt1 isolines were developed using PI 83.945-4 (from Korea), PI 84.987 ('Oni hadaka' from Japan), and PI 86.024 ('Daizuhinshu satei' from Japan) as donors and all three were quite short. More recently we developed isolines using Group IV cv. Peking (introduced from China) and Group I cv. Soysota (introduced from Italy) as donors of what we thought was dt1 and obtained BC5 isolines that were about 50% taller than the previous dt1 isolines. We then undertook genetic studies to discover if this difference was due to modifying genes or to the presence of some gene other than dt1

Table 1 lists the BC5 Clark isolines used in this study. The two isolines with Peking and Soysota as the donors of determinate stem are taller than the dt1 isoline using PI 84.987 and only slightly different in appearance from the semideterminate Clark isoline with gene Dt2. They are also taller than the Clark isoline combining determinate stem and long internode (dt1 s-t).

Results, Discussion, and Conclusions: The two tall determinate isolines were crossed and an F2 population of over 300 plants from 3 different F1 plants was observed to have a uniform plant type similar to the parents. We concluded that L91-8060 (Peking donor) and L91-8052 (Soysota donor) have the same genotype. We observed the F2 and F3 of Clark x L91-8060 and Clark x L91-8052. Results indicating monogenic segregation based on F3 observations are presented in Table 2. There were no short determinate plants observed in either F2 or F3. In the F2 generation we classified plants into three categories: indeterminate, intermediate, and tall determinate. It was difficult to discriminate between individual indeterminate (Dt1 Dt1) and intermediate (Dt1 dt1) plants, and overall about 20% were misclassified; whereas all but one F2 plant classed as tall determinate were homozygous giving true-breeding progenies. We concluded from these results, that the tall determinate phenotype was caused by a single gene pair and that the heterozygote was intermediate but closer to the indeterminate phenotype.

We crossed L62-1251 (Dt2 isoline) x L91-8060 (Peking donor) and in the F2 found 75 determinate plants and 4 indeterminate ones, a very good fit to the expected 15:1 ratio (chi-square probability = .63), demonstrating that the tall determinate gene is not located at the Dt2 locus. We crossed short determinate L63-3297 with L91-8060 and found the F2 and F3 to be all determinate. We classified 32 F3 families, observing 30 to 40 plants in each family, and found 6 all short, 21 segregating, and 5 all tall families, a reasonable fit to a 1:2:1 ratio (Chi-square probability = .074). F2 stands were poor and plant heights therefore somewhat irregular, but with one exception the homozygous tall F2 plants ranged between 53 and 62 cm and the heterozygous F2 plants ranged from 33 to 53 cm (with 6 very short, perhaps damaged, exceptions). The homozygous short F2 plants ranged from 6 to only 11 cm (except for 1 much taller, probably misidentified exception). Excluding the 8 aberrant F2 plants, heights averaged 58, 45, and 10 cm for the 3 genotypes. We conclude that the gene causing the tall determinate phenotype was allelic to dt1 and that the heterozygote is intermediate but closer to the tall homozygote.

We observed F2 populations of over 300 plants each, of L91-8060 crossed with the determinate cultivars Spry, Ripley, and Hobbit 87. All three populations were all-determinate but there was considerable segregation for maturity and probably other growth-affecting factors. Nevertheless we found short determinate segregates in each cross. Although a complete classification was not attempted we believe that these 3 cultivars carry the short determinate allele. The dt1 allele in Hobbit 87 and Ripley comes from southern cultivars Ransom and York, respectively. Spry has several potential sources of dt1 in its pedigree.

We also crossed the Clark dt1 s-t isoline (determinate and long internode) with the tall determinate isoline from Peking (L67-3207 x L91-8060) and found all determinate plants in the F2 and F3. Among 56 F3 lines we found three (expected 3.5) that were true-breeding short (comparable to L63-3297) and several that were uniformly taller than the L91-8060 parent showing that s-t is not a factor in the tallness of L91-8060.

In a previous study Bernard (1972) reported that several southern U.S. and Japanese cultivars as well as Ebony and Peking all had the same dt1 gene. This was based on observation of F2 populations which were all determinate. Since these populations were segregating for time of flowering and maturity and presumably many other growth-affecting gene, all differences in plant height were assumed to be caused by these genes, and the tall determinate allele was not identified until we observed its effect in an isogenic background.

It is interesting to note that Woodworth (1933) in the original paper on dt1 named Peking as the cultivar typical of the determinate trait, but the inheritance data presented involved only Ebony. Since the dt1 allele was named based on segregation in the cross of Manchu x Ebony, a gene symbol cannot be assigned to the third allele until we know which allele Ebony carries. Its tall determinate phenotype could easily be due to the same allele as in Peking and Soysota (which means we will need a new symbol for the short determinate allele), or it could have the short determinate allele with modifiers making it tall. Crosses have been made with Ebony and the third allele will be symbolized when we publish these results.

References:

Bernard, R. L. 1972. Two genes affecting stem termination in soybeans. Crop Sci. 12:235-239.

Bernard, R. L., R. L. Nelson, and C. R. Cremeens. 1991. USDA Soybean Genetic Collection: Isoline Collection. Soybean Genetics Newsltr. 18:27-57.

Johnson, H. W. 1958. Registration of soybean varieties, VI. Agron. J. 50:690-691.

Woodworth, C. M. 1933. Genetics of the soybean. J. Amer. Soc. Agron. 25:36-51.

J. A. Thompson, University of Illinois
R. L. Bernard, University of Illinois
R. L. Nelson, USDA-ARS and University of Illinois

Table 1. Parents of crosses used to study tall determinate genetics and plant heights measured at maturity in 1993.

Strain	Transferred Genes	Parentage	Stem Type	Ht*
Clark (Dt1 dt2 s)		Lincoln(2) x Richland	Indeterminate	110
L63-3297	dt1?	Clark(6) x PI 84.987	Determinate	49
L62-1251	Dt2	Clark(6) x T117	Semi-det.	86
L67-3207	dt1?,s-t	(Clark(6) x Chief) x L63-3297	Determinate	63
L91-8052	dt1?	L6(6)# x Soysota	Tall det.	83
L91-8060	dt1?	L6(6)# x Peking	Tall det.	85

* Plant heights in centimeters taken at maturity are the mean of twenty plants measured from each of four replications.

L6 (Bernard et. al. 1991) is a phytophthora and bacterial pustule resistant isoline of Clark similar if not identical to Clark 63. No effects from these two diseases were observed during this study.

Table 2. Segregation in populations of Clark crossed with two tall determinate isolines. F2 genotype based on observation of F3. Expected values in parentheses.

Cross	Clark x L91-8060	Clark x L91-8052
	number of F2 plants	
All Indeterminate	15 (14.8)	36 (40.8)
Indeterminate & Tall determinate	30 (29.5)	85 (81.5)
All Tall determinate	14 (14.8)	42 (40.8)
Expected ratio	1:2:1	1:2:1
Chi-square probability	0.97	0.69

CLEMSON UNIVERSITY
Department of Agronomy and Soils
Poole Agricultural Center Box 340359
Clemson, SC 29634-0359

Photomorphogenic influence of light quality on expression of the long-juvenile trait in soybean

Introduction: In 1979, Hartwig and Kiihl described a delayed flowering character in PI 159925 soybean. The trait was subsequently used in cultivar development and termed "long-juvenile" (Hinson, 1989). In addition to delayed flowering under short photoperiod conditions, several reports indicate that the long-juvenile (LJ) trait also influences morphological development (Tomkins et al., 1993, Board and Settimi, 1986). Although the influence of photoperiod on expression of the LJ trait has been documented (Sinclair and Hinson, 1992), the photomorphogenic influence of light quality has not been adequately characterized. In soybean, light quality is known to influence the timing and sensitivity of the flowering response at the E_3, e_3 locus (Buzzell, 1971; Kilen and Hartwig, 1971) and internode length at the Sb_1, sb_1 and Sb_2, sb_2 loci (Huang et al., 1993). Since light quality controls the expression of other developmental traits in soybean, a growth chamber study was undertaken to evaluate floral and morphological responses of LJ near-isoline pairs to three different light quality regimes.

Materials and methods: Four LJ near-isoline pairs (F85-1107/1108, F85-1173/1182, F85-372/369-1, and F85-1020/1027) were established in 1000 ml pots containing soil in which soybeans had been grown previously. Normal isolines F85-1107 and F85-1173 were maturity group III types while F85-372 and F85-1020 were maturity group VI types. The soil was a Riverview loam (fine-loamy, mixed, thermic Fluventic Dystrochrepts) fertilized with 33.6 kg ha^{-1} of P and 67.2 kg ha^{-1} of K, and pH was 6.5. After emergence, the plants were thinned to three plants per pot. Different red : far-red (R : FR) light quality levels were conducted under an inductive 11 h photoperiod utilizing the following artificial light sources: (i) fluorescent (R enriched), (ii) fluorescent/incandescent (R / FR), and (iii) incandescent (FR enriched). The same growth chamber was utilized for all light quality treatments, so treatments were separated in time. Growth chamber temperature was maintained at 25/22 °C during the day/night cycle. At first flower (R1 stage), individual plants were harvested, and plant

height (cotyledonary node to terminal bud), number of main stem nodes (excluding cotyledonary node), and leaf area were determined. Days to R1 from emergence were also determined.

The experimental design was a completely randomized design with two factors (genotype and light quality treatment) and six replications. Data were analyzed with SAS (Cary, NC) using general linear model procedures. Means were separated using the LSD method at the 0.05 probability level.

Results and Discussion: Across genotypes, average days to flower was 42 d for FR conditions and 32 d for the R and R/FR environments. Additionally, the delay in flowering within isoline pairs conferred by the LJ trait was greatest under FR conditions. This response was characterized by a greater increase in days to flower for LJ lines under FR conditions. These results suggest that the level of FR light influences the timing of floral initiation differently for normal and LJ soybean genotypes.

Across all light quality treatments, the LJ lines exhibited greater levels of growth and development for each morphological parameter compared to the normal lines. These responses were most likely the result of an extension of the vegetative growth cycle conferred by the LJ trait. Magnitude of leaf area response to light quality for all genotypes was R/FR > R > FR. However, magnitude of leaf area differences within isoline pairs was R > R/FR > FR. This response was characterized by a higher level of leaf area growth in response to R light for the LJ genotypes. Magnitude of plant height response to light quality for 7 of the 8 genotypes was R/FR > R > FR. Although one isoline pair (F85-1107/1108) exhibited a relatively consistent plant height response across light quality treatments, magnitude of differences within the remaining 3 pairs was R > R/FR > FR, following the same pattern as leaf area. Number of main stem nodes was similar between R and R/FR environments for 7 of the 8 genotypes. In the FR environment, node number decreased for all genotypes. There was no general trend within isoline pairs for differences in main stem node number between light quality treatments. Average internode length (plant height / number of main stem nodes) was shortest in the R environment and longest in the R/FR and FR environments. Increased internode length in response to FR light is a commonly observed phenomenon (Ballare et al., 1990, Kasperbauer, 1971). As with node number, there was no general trend within isoline pairs for differences in internode length between light quality treatments.

Conclusion: Based upon these preliminary results, expression of the long-juvenile trait as influenced by light quality appears to differ from the normal soybean response under inductive photoperiods. Although both LJ and normal isolines were influenced by light quality in a similar manner, the magnitude of response varied between LJ and normal genotypes for days to flower, leaf area, and plant height characteristics. The distribution and reflectance of R and FR light within the plant canopy affects plant growth and development (Ballare et al., 1990, Kasperbauer, 1971). Therefore, under short-day conditions, LJ and normal soybean genotypes may respond differently to environmental factors and production practices that alter R : FR light ratios.

Acknowledgment: Appreciation for the germplasm used in this study is extended to Kuell Hinson, University of Florida, Gainesville.

Table 1. Days to flower (DTF) and leaf area of LJ soybean isolines grown under red enriched (R), combined R / FR (R/FR), and FR enriched (FR) 11 h photoperiods. Data taken at the R1 stage of development.

Isoline pair†	Days to flower				Leaf area			
	R	R/FR	FR	LSD	R	R/FR	FR	LSD
	plant^{-1}				cm^{-2} plant^{-1}			
F85-1108 (LJ)	32	29	41	1	163	210	76	21
F85-1107 (NJ)	26	25	32	1	118	172	53	18
Δ	6*	4*	9*		45*	38*	23*	
F85-1182 (LJ)	38	36	51	1	258	291	111	22
F85-1173 (NJ)	28	29	39	1	151	209	52	21
Δ	10*	7*	12*		107*	82*	59*	
F85-369-1 (LJ)	39	38	49	1	268	317	73	25
F85-372 (NJ)	28	28	34	1	126	208	51	12
Δ	11*	10*	15*		142*	109*	22*	
F85-1027 (LJ)	39	38	52	1	323	376	151	39
F85-1020 (NJ)	30	33	41	1	169	265	82	28
Δ	9*	5*	11*		154*	111*	69*	

* Difference between isolines is significant at the 0.05 level.
† Long-juvenile (LJ), normal-juvenile (NJ).

Table 2. Plant height and internode length of LJ soybean isolines grown under red enriched (R), combined R / FR (R/FR), and FR enriched (FR) 11 h photoperiods. Data taken at the R1 stage of development.

Isoline pair†	Plant height				Main stem nodes			
	R	R/FR	FR	LSD	R	R/FR	FR	LSD
	cm plant^{-1}				No. plant^{-1}			
F85-1108 (LJ)	63	78	49	7	6.3	6.7	3.7	0.6
F85-1107 (NJ)	40	52	29	6	5.0	5.5	3.3	0.5
Δ	23*	26*	20*		1.3*	1.2*	4.0*	
F85-1182 (LJ)	84	81	60	6	7.3	7.0	4.2	0.5
F85-1173 (NJ)	31	40	34	5	4.8	5.0	3.2	0.4
Δ	53*	41*	26*		2.5*	2.0*	1.0*	
F85-369-1 (LJ)	75	92	30	13	8.0	8.0	3.8	0.5
F85-372 (NJ)	33	61	25	9	5.2	6.0	3.8	0.9
Δ	42*	31*	5*		2.8*	2.0*	0.0	
F85-1027 (LJ)	86	102	54	11	8.2	7.7	5.2	0.6
F85-1020 (NJ)	44	77	35	10	5.0	7.0	3.2	0.7
Δ	42*	25*	19*		3.2*	0.7*	2.2*	

* Difference between isolines is significant at the 0.05 level.
† Long-juvenile (LJ), normal-juvenile (NJ).

References:

Ballare, C.L., A.L. Scopel, and R.A. Sanchez. 1990. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. Science 247:329-332.

Board, J.E., and J.R. Settimi. 1986. Photoperiod effect before and after flowering on branch development in determinate soybean. Agron. J. 78:995-1002.

Buzzell, R.I. 1971. Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. 13:703-707.

Hartwig, E.E., and A.S. Kiihl. 1979. Identification and utilization of a delayed flowering character in soybeans for short-day conditions. Field Crops Res. 2:145-151.

Hinson. K. 1989. Use of a long-juvenile trait in cultivar development. p. 983-987. *In* A.J. Pascale (ed.) Proc. World Soybean Res. Conf. IV. Buenos Aires, Argentina. 5-9 Mar. 1989. Argentina Soybean Assoc., Buenos Aires.

Huang, S., D.A. Ashley, and H.R. Boerma. 1993. Light intensity, row spacing, and photoperiod effects on expression of brachytic stem in soybean. Crop Sci. 33:29-37.

Kasperbauer, M.J. 1971. Spectral distribution of light in a tobacco canopy and effects of end-of-day light quality on growth and development. Plant Physiol. 47:775-778.

Kilen, T.C. and E.E. Hartwig. 1971. Inheritance of a light quality sensitive character in soybean. Crop Sci. 11:559-561.
Sinclair, T.R., and K. Hinson. 1992. Soybean flowering in response to the juvenile trait. Crop Sci. 32:1242-1248.
Tomkins, J.P., E.R. Shipe, and K. Hinson. 1993. Growth and developmental responses of long-juvenile soybean genotypes at early and conventional planting dates. Soybean Gen. Newsl. 20:168-172.

Jeff P. Tomkins
Emerson R. Shipe
Susan U. Wallace

CLEMSON UNIVERSITY
Department of Agronomy and Soils
Poole Agricultural Center Box 340359
Clemson, SC 29634-0359

Influence of light quality on photomorphogenic responses of delayed flowering soybean genotypes

Introduction: There is current interest in developing and utilizing soybean genotypes that contain a delayed flowering trait which would allow early and staggered plantings in the southern U.S.A. However, the physiological response of such genotypes to important environmental components such as light quality is not well characterized. Plant growth and development are influenced by the distribution and reflectance of red (R) and far-red (FR) light within the plant canopy (Ballare et al., 1990; Kasperbauer, 1971). Therefore, it is important to understand how agronomic plant species respond to changes in the R : FR light ratio. In soybean, light quality is known to influence the timing and sensitivity of the flowering response at the E_3, e_3 locus (Buzzell, 1971; Kilen and Hartwig, 1971) and internode length at the Sb_1, sb_1 and Sb_2, sb_2 loci (Huang et al., 1993). However, other developmental soybean traits have not been characterized for light quality response. Since light quality controls the photomorphogenic expression of floral and stem structural traits in soybean, a growth chamber study was undertaken to evaluate floral and morphological responses of different delayed flowering soybean genotypes to three different light quality regimes.

Materials and methods: Nine soybean genotypes, four of which have been characterized as delayed flowering genotypes (Board and Settimi, 1986; Hartwig and Kiihl, 1979) were established in 1000 ml pots containing a Riverview loam (fine-loamy, mixed, thermic Fluventic Dystrochrepts) in which soybeans had been grown previously. The soil was fertilized with 33.6 kg ha^{-1} of P and 67.2 kg ha^{-1} of K, and pH was 6.5. After emergence, the plants were thinned to three plants per pot. Different R : FR light ratios were conducted under a noninductive 15 h photoperiod utilizing the following artificial light sources: (i) fluorescent (R enriched), (ii) fluorescent/incandescent (R / FR), and (iii) incandescent (FR enriched). Treatments (i) and (ii) were previously used by Kilen and Hartwig (1972) to characterize the E_3, e_3 locus. The same growth chamber was utilized for all light quality treatments, so treatments were separated in time. Growth chamber temperature was maintained at 25/22 °C during the day/night cycle. At 40 d from emergence, the plants were harvested, and plant height (cotyledonary node to terminal bud) and number of main stem nodes (excluding

cotyledonary node) were determined. Plants were also observed daily for time of flowering (R1 stage). The experimental design was a completely randomized design with two factors (genotype and light quality treatment) and six replications. Data were analyzed with SAS (Cary, NC) using general linear model procedures. Means were separated using the LSD method at the 0.05 probability level.

Results and discussion: Unlike Dorman and Arksoy, none of the other delayed flowering types exhibited sensitivity to fluorescent light at the E3/e3 locus (Table 1). In regards to stem and node development, the delayed flowering types were generally not different than the conventional types for each light quality regime (Tables 2 and 3). This response supports previous observations of similar morphological development of field grown conventional and delayed flowering soybean genotypes under noninductive photoperiods associated with late-May plantings (Tomkins et al., 1993).

Overall plant height and internode length increased as the level of FR light was increased (Table 2). This response is in good agreement with previous findings for plant response to changes in light quality (Kasperbauer, 1971; Ballare et al.,1990). Average plant heights were 62.5 (R enriched), 82.4 (R / FR), and 104.6 (FR enriched) cm, and internode lengths were 8.9, 9.8, and 17.4 cm, respectively. In comparison to the other genotypes, Arksoy and Davis exhibited a 49% reduction in height and a 45 % reduction in internode length in the R enriched environment. The unusual response was still expressed in the R / FR environment but, disappeared under FR enriched conditions as plant height and internode length were generally the same for all genotypes. In a report on the brachytic stem trait, Boerma and Jones (1978) proposed the Davis genotype ($Sb_1Sb_1Sb_2Sb_2$) to be different than other normal stemmed genotypes ($Sb_1Sb_1sb_2sb_2$). Therefore, the short internode response under R light conditions may be related to the Sb_2, sb_2 locus.

Conclusion: Varying the R : FR light ratio under noninductive photoperiod conditions did not reveal any unusual light sensitivity responses in delayed flowering soybean genotypes compared to conventional types. However, a unique R light dependent response affecting plant height and internode length in the soybean cultivars Arksoy and Davis was identified. Since Arksoy is ancestral to Davis, modern cultivars with a Arksoy / Davis background are being screened for the short internode response to determine if plant breeders have indirectly selected for the trait. In order to determine the genetic basis of the trait, studies have been undertaken to characterize inheritance and the relationship of the response to the Sb_2, sb_2 locus.

Table 1. Maturity group, flowering characteristics, and days to flower (DTF) of soybeans grown under red enriched (R), combined R / FR (R/FR), and FR enriched (FR) 15 h photoperiods.

Genotype	Maturity Group	DF type†	DTF		
			R	R/FR	FR
PI 285096	X	?	*	*	*
Santa Maria	VIII	yes	*	*	*
Padre	VII	yes	*	*	*
PI 159925	VI	yes	*	*	*
Hill	V	?	*	*	*
Dorman	V	no	30	*	*
Lee	VI	no	*	*	*
Arksoy	VI	no	29	*	*
Davis	VI	yes	*	*	*

* No floral buds observed 40 d after emergence.
† Genotype exhibits a delayed flowering response characteristic (DF type).

Table 2. Plant height and internode length of soybeans grown under red enriched (R), combined R / FR (R/FR), and FR enriched (FR) 15 h photoperiods.

Genotype	Plant height				Internode length†			
	R	R/FR	FR	LSD	R	R/FR	FR	LSD
	cm plant^{-1}				cm internode^{-1}			
PI 285096	79.0	91.8	112.7	16.0	12.2	10.4	16.8	1.8
Santa Maria	66.2	98.7	112.2	27.5	11.0	11.3	19.8	3.0
Padre	70.7	79.2	97.5	23.2	12.5	10.6	18.8	1.0
PI 159925	68.0	84.8	109.3	12.6	11.6	10.4	16.8	1.2
Hill	77.0	93.8	88.8	11.5	11.7	10.4	16.7	2.0
Dorman	61.7	97.8	101.0	14.8	10.6	12.2	16.8	1.5
Lee	68.5	89.8	99.0	17.3	11.1	10.4	17.0	2.3
Arksoy	36.0	57.2	112.7	10.7	6.6	6.8	16.4	1.3
Davis	35.8	48.0	108.0	13.7	6.1	6.0	17.5	1.4
LSD (0.05)	12.1	13.0	NS		1.9	1.4	2.0	

† Internode length = plant height / no. nodes.

References:

Ballare, C.L., A.L. Scopel, and R.A. Sanchez. 1990. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. Science 247:329-332.

Board, J.E., and J.R. Settimi. 1986. Photoperiod effect before and after flowering on branch development in determinate soybean. Agron. J. 78:995-1002.

Boerma, H.R., and B.G. Jones. 1978. Inheritance of a second gene for brachytic stem in soybean. Crop Sci. 18:559-560.

Buzzell, R.I. 1971. Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. 13:703-707.

Hartwig, E.E., and A.S. Kiihl. 1979. Identification and utilization of a delayed flowering character in soybeans for short-day conditions. Field Crops Res. 2:145-151.

Huang, S., D.A. Ashley, and H.R. Boerma. 1993. Light intensity, row spacing, and photoperiod effects on expression of brachytic stem in soybean. Crop Sci. 33:29-37.

Kasperbauer, M.J. 1971. Spectral distribution of light in a tobacco canopy and effects of end-of-day light quality on growth and development. Plant Physiol. 47:775-778.

Kilen, T.C. and E.E. Hartwig. 1971. Inheritance of a light quality sensitive character in soybean. Crop Sci. 11:559-561.

Tomkins, J.P., E.R. Shipe, and K. Hinson. 1993. Growth and developmental responses of long-juvenile soybean genotypes at early and conventional planting dates. Soybean Gen. Newsl. 20:168-172

Jeff P. Tomkins
Emerson R. Shipe
Susan U. Wallace

IOWA STATE UNIVERSITY
Department Of Zoology And Genetics
Ames, IA 50011 USA

An estimation of the physical distance between co-segregating RFLP markers in the soybean genome

It would be very helpful to know the relationship between genetic distance and physical distance in the soybean genome to aid in marker-based cloning, especially due to its large size and the high percentage of repetitive sequences (1, 2). To obtain an answer to this question, we have begun experiments where we sequentially hybridize two or more closely linked probes to the same blot of electrophoretically separated DNA fragments. Pairs of probes which hybridize at the same DNA band are likely to be present on the same DNA fragment. By using a series of different restriction endonucleases, which yield different sized fragments, we have reduced the probability that two probes hybridize to different but identically sized fragments. Using this approach, an analysis of a large number of closely linked genetic markers should yield a correlation between average physical distance and genetic distance in the area of the genome where RFLP probes are selected.

Materials and Methods: [Glycine max (L) Williams] plants were grown in the greenhouse. Young expanding trifoliate leaves or primary leaves were utilized for the preparation of protoplasts. Intact chromosomal DNA was prepared by a previously published procedure (3) and yielded high molecular weight DNA. DNA embedded in agarose plugs was digested with restriction endonucleases for 6 hours under conditions recommended by the manufacturer (New England Biolabs). Electrophoresis conditions (CHEF, Biorad) were according to the desired range of size resolution. Yeast chromosomes prepared according to Schwartz and Cantor (4) and concatermers of lambda DNA imbedded in agarose were used as size markers. Following electrophoretic separation of DNA fragments, DNA was treated with UV light and transferred to nylon membranes (GeneScreen Plus, Du Pont Corp.) and hybridized to ^{32}P-labeled DNA probes of high specific activity (0.5-2 x 10^9 cpm/ug DNA) in a sequential manner, removing the previously labeled DNA by two 10 minute washes in 0.1 SSC, 0.1% SDS at 95^{o}C. Re-exposure to X-ray film for 3-6 days confirmed the absence of residual label. RFLP probes were selected from the USDA-ARS, Iowa State University map (5). All closely linked probes were first hybridized to each other to eliminate the possibility that they were overlapping sequences. Of the

11 pairs of probes tested, three were found to hybridize to each other and were not pursued further.

Results and Discussion: The soybean RFLP map now contains about 450 polymorphic markers, spanning about 3100 centimorgans. During the generation of this map, a number of randomly selected probes were found to map at the same locations. However, with the relatively small number of progeny (58) in the F_2 generation to estimate the map distance, markers would need to be located approximately 2.5 cM apart before being 95% certain of detecting a recombination event. Consequently, two co-segregating markers may be physically separated by a large amount of DNA. A problem with the interpretation of sequential hybridization data comes from the fact that at a particular band site more than one fragment may be present; one which hybridizes to one probe and the second which hybridizes to the second probe. This complication has been minimized by digesting the DNA with several different restriction endonucleases followed by sequential hybridizations to each digest.

TABLE 1. CO-HYBRIDIZATION OF CLOSELY LINKED RFLP PROBES

Linked Probes	Linkage group	Genetic distance	Digesting enzyme	Co-migrating bands (kb)
pA-183 & pA-427	B	0	*Mlu*I	370
				200
			*Hind*III	21.4
pA-118 & pA-520	B	0	*Mlu*I	158
			*Pst*I	154
			*Eco*RI	10.5
pA-73 & pA-584	G	0	*Mlu*I	251
			*Eco*RI	14.0
			*Bgl*II	11.5
pA89 & pK9	H	0	*Sfi*I	470
			*Not*I	430
				360
			*Mlu*I	240
			*Hind*III	14
pA-106 & pA-450	L	0	*Eco*RI	4.1
			*Mlu*I	none
			*Bgl*II	none
			*Sfi*I	none
pK-474 & pA-538	C	0	*Mlu*I	158
			*Eco*RI	9.5
			*Bgl*II	12.6
pA-104 & pA-170	A	0.9	*Eco*RI	11.2
			*Hind*III	none
			*Mlu*I	none
			*Bgl*II	none
pA-404 & pA-427	B	1.0	*Mlu*I	200
			*Sfi*I	480
pA-404 & pA-183	B	1.0	*Mlu*I	200

From the data presented in Table 1, all but one pair of the co-segregating probes reveal relatively small-sized sequentially hybridizing fragments (about 10-20 kb). One of the co-segregating probes did not give any sequential hybridization as did one pair of probes that map 0.90 cM apart, suggesting they are more than 400 kb apart. Although one common band was found for probe pairs pA-106/pA-450 and pA-104/pA-170, it is unlikely that these small EcoRI bands represents the same DNA fragment since no larger co-migrating band was found with the other enzymes. The two pairs of probes that map 1 cM apart both gave minimum distances of about 200 kb, much less than expected.

These data suggest that co-segregating RFLP markers may be physically much closer than anticipated and are encouraging for the prospects of marker-based cloning. On the other hand, they do raise interesting questions as to why such a large number of closely linked markers are physically so close. With the size of the soybean genome being near 1.5×10^9 bp and the genetic map of 3100 cM, the average physical distance should correspond to about 500 kb/cM. One possible explanation for these unexpected results is that anomalous recombination frequencies are being observed in these regions of the genome. In addition, RFLP probes are not randomly selected and may bias the results. Probes that generate a large number of bands are discarded as well as probes that do not generate polymorphisms. The selection of probes may be biased to certain areas of the genome, for example regions lacking repetitive DNA.

References:

Bennett, M.D., J.B. Smith, and J.S. Heslop-Harrison. 1982. Nuclear DNA amounts in angiosperms. Proc. Roy. Soc. Lond. 126:179-199.

Goldberg, R.B. 1978. DNA sequence organization in the soybean plant. Biochem. Genet. 16:45-68.

Honeycutt, R.J, B.W.S Sobral, M. McClelland, and A.G. Atherly. 1992. Analysis of large DNA from soybean (Glycine max (L.) Merr.) by pulsed-field gel electrophoresis. The Plant J. 2:133-135.

Schwartz, D.C. and C.R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37:67-75.

Diers, B.W., L. Mansur, J. Imsande, and R. Shoemaker. 1992. Mapping of Phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. 32:377-383.

Duan Xiaozhu
Randy Shoemaker, USDA-ARS-FCR
Alan Atherly

UNIVERSITY OF MARYLAND
Department of Agronomy
College Park, MD 20742
and
ATOMIC ENERGY AUTHORITY
Radiobiology Department
Cairo, Egypt

Salt Tolerance of Soybean in Solution Culture Experiments. I.Evaluation of Screening Technique

Introduction: Soil salinity is a widespread problem affecting more than 955 million hectares worldwide (Szabolcs, 1989). Researchers have identified cultivars of soybean that exhibit salt tolerance, however nearly all are southern-adapted lines (Parker et al., 1986; Yang and Blanchar, 1993). The purpose of our experiments was to identify salt-tolerant soybean cultivars adapted to the Mid-Atlantic growing region. This paper is the first of two consecutive papers presenting the results of those studies.

Materials and Methods: Soybean lines 'Clark', 'Cutler-71', 'Jackson', and 'Lee', and MD71-583 were evaluated for salt tolerance. Jackson and Lee are known to be salt-sensitive and salt-tolerant, respectively (Abel and Mackenzie, 1964; Parker et al., 1986). Five seedlings of each genotype were suspended by styrofoam Todd planter flats in 15 L of nutrient solution (modified from Johnson et al., 1957) in plastic containers and allowed to grow for 21 days. At that time, salt treatments with electrical conductivity values of 2.0, 6.0, 11.0, and 16.0 dS m^{-1}, respectively, were imposed for 14 days. Shoot fresh weight was measured and shoot injury evaluated by a visual chlorosis score (0 = healthy, 5 = dead).

Results and Discussion: There were significant genotype differences for shoot fresh weight at all salt levels, including the control. Data expressed as percent of control therefore provides a relative appraisal of differences among the genotypes (Table 1). Lee produced more than twice the relative shoot fresh weight of all other genotypes at the 6.0 dS m^{-1} salt level, but suffered relative reductions in shoot fresh weight similar to that of the other cultivars at salt levels higher than 6.0 dS m^{-1}. Lee was significantly lower in

chlorosis score than all other genotypes at the 6.0 dS m^{-1} salt treatment, but substantial chlorotic symptoms became evident for this cultivar at 11.0 and 16.0 dS m^{-1} salt levels (Table 2). Of the genotypes tested, only Lee appeared to express salt tolerance, and tolerance was limited to salt concentrations which had electrical conductivity values less than 11.0 dS m^{-1}. Experiment II was therefore initiated to evaluate many more soybean cultivars at salt levels up to 10.9 dS m^{-1}.

References:

Abel, G.H., and A.J. MacKenzie. 1964. Salt tolerance of soybean varieties (Glycine max L. Merill) during germination and later growth. Crop Sci. 4:157-161.

Johnson, C. M., P. R. Stout, T. C. Broyer, and A. B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant Soil (VIII) 4:337-353.

Parker, M.B., T.P. Gaines, and G.J. Gascho. 1986. Sensitivity of soybean cultivars to soil chloride. Georgia Agric. Exp. Sta. Res. Bull. 347.

Szabolcs, I. 1989. Salt-affected soils. CRC Press. Boca Raton, FL.

Yang, J. and R.W. Blanchar. 1993. Differentiating chloride susceptibility in soybean cultivars. Agron. J. 85:880-885.

A.S. Ragab*
V.R. Pantalone III
W.J. Kenworthy
B.R. James

* visiting lecturer

Table 1. Shoot fresh weight of five soybean genotypes grown 14 days in solution culture with NaCl treatments of 2, 6, 11, and 16 dS m^{-1}.

	NaCl Treatment			
	dS m^{-1}			
	2.0	6.0	11.0	16.0
	Shoot fresh weight (percent of control)			
Clark	1.00	0.27	0.31	0.16
Cutler 71	1.00	0.28	0.27	0.15
Jackson	1.00	0.23	0.20	0.13
Lee	1.00	0.58	0.26	0.20
MD71-583	1.00	0.27	0.31	0.17

Table 2. Chlorosis score of five soybean genotypes grown 14 days in solution culture with NaCl treatments of 2, 6, 11, and 16 dS m^{-1}.

	NaCl Treatment			
	dS m^{-1}			
	2.0	6.0	11.0	16.0
	Score			
Clark	0.0	3.8	4.6	5.0
Cutler 71	0.0	3.6	4.5	4.7
Jackson	0.0	3.6	4.4	5.0
Lee	0.0	0.5	2.5	3.1
MD71-583	0.0	2.0	2.6	4.2
LSD(0.05)	0.3	0.3	0.3	0.3

Score (0 = healthy, 5 = dead)

Salt Tolerance of Soybean in Solution Culture Experiments.

II. Reaction of 19 Genotypes

Introduction: In the previous paper, we found that of five soybean genotypes tested, only one, 'Lee' expressed salt tolerance in solution culture at salt treatments less than 11.0 dS m^{-1}. The present experiment will examine several additional soybean lines at salt concentrations up to 10.9 dS m^{-1}. Chloride analyses of plant tissue will aid in identifying salt-tolerant soybean germplasm.

Materials and Methods: Soybean lines 'Avery', 'Bay', 'Clark', 'Cutler71', 'Douglas', 'Essex', 'Forrest', 'Jackson', 'Lee', 'MD71-583', 'Morgan', 'Pella', 'Pyramid', 'Regal', 'Ripley', 'Stafford', 'Toano', 'Williams', and 'Zane' were evaluated for salt tolerance in aquaria with 45 L nutrient solution. The nutrient solution was modified from Johnson et al. (1957). Salt treatments with electrical conductivity values of 2.0, 4.5, 7.5, and 10.9 dS m^{-1} were imposed for 14 days after seedlings had been allowed to grow for 21 days. Chloride concentration was measured from shoot tissue using the procedure of Florence and Farrar (1971). Shoot fresh weight, dry weight, and height were measured.

Results and Discussion: When averaged over all 19 genotypes, increased NaCl concentration resulted in significantly increased shoot chloride concentration and there was a trend for reduced shoot fresh weight, dry weight, and height. This is consistent with the findings of other soybean researchers (Abel and Mackenzie, 1964; Parker *et al.*, 1986, Yang and Blanchar, 1993).

Table 1 shows shoot chloride concentration with the LSD value for comparing genotypes within a salt treatment, and shoot fresh weight, dry weight, and height expressed as a percent of the control. Data for the control treatment (2.0 dS m^{-1}) is not presented. In the control treatment, there were no significant differences among genotypes for chloride concentration (genotype mean = 2.1 mg g^{-1} Cl), and shoot fresh weight, dry weight, and height were 100%.

Chloride exclusion in soybean is governed by a single gene (Abel, 1969). Therefore shoot chloride concentration is a good indicator of tolerance or sensitivity to salt. Table 1 is ranked by chloride concentration at the 7.5 dS m^{-1} treatment because this level of salt showed a differential response among genotypes. In all three NaCl treatments, the group Avery, Lee, Morgan, and Pella were significantly lower in shoot

chloride concentration in contrast to the remaining genotypes. These four cultivars may be considered as chloride excluders.

At the highest salt treatment (10.9 dS m^{-1}), no genotype had a chloride concentration that was significantly lower than that of the salt-sensitive cultivar Jackson. This suggests that there may be some upper threshold limit for salt tolerance in soybean, governed by the chloride exclusion mechanism. However, Morgan was significantly lower in shoot chloride concentration than most of the other genotypes at the highest salt treatment level. Moreover, Morgan excluded chloride, and maintained relatively high shoot fresh weight, dry weight and height for every salt treatment level. The salt tolerance of this cultivar should be further investigated.

References:

Abel, G.H. 1969. Inheritance of the capacity for chloride inclusion and chloride exclusion in soybeans. Crop Sci. 9:697-698.

Abel, G.H., and A.J. MacKenzie. 1964. Salt tolerance of soybean varieties (Glycine max L. Merill) during germination and later growth. Crop Sci. 4:157-161.

Florence, T.M., and Y.J. Farrar. 1971. Spectrophotometric determination of chloride at the parts-per-billion level by the mercury (II) thiocyanate method. Anal. Chim. Acta. 54:373-377.

Johnson, C. M., P. R. Stout, T. C. Broyer, and A. B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant Soil (VIII) 4:337-353.

Parker, M.B., T.P. Gaines, and G.J. Gascho. 1986. Sensitivity of soybean cultivars to soil chloride. Georgia Agric. Exp. Sta. Res. Bull. 347.

Yang, J., and R.W. Blanchar. 1993. Differentiating chloride susceptibility in soybean cultivars. Agron. J. 85:880-885.

A.S. Ragab*
V.R. Pantalone III
W.J. Kenworthy
B.R. James

* visiting lecturer

Table 1. Chloride concentration and shoot fresh weight, dry weight, and height (percent of control) of 19 soybean genotypes grown in solution culture 14 days at 4.5, 7.5, and 10.9 dS /m.

	NaCl Trt (dS.m)			NaCl Trt (dS/m)			NaCl Trt (dS/m)			NaCl Trt (dS/m)		
	4.5	7.5	10.9	4.5	7.5	10.9	4.5	7.5	10.9	4.5	7.5	10.9
	Shoot Chloride mg/g			Shoot Fresh Weight (percent of control)			Shoot Dry Weight (percent of control)			Shoot Height (percent of control)		
Morgan	2.7	7.8	20.3	1.05	0.91	0.85	0.91	0.69	0.70	0.96	0.86	0.83
Avery	2.0	11.8	30.4	1.19	0.79	0.30	1.02	0.56	0.24	0.92	0.78	0.55
Lee	2.7	12.5	29.0	0.99	0.50	0.39	0.95	0.40	0.39	0.92	0.71	0.64
Pella	3.6	14.1	31.2	0.84	0.76	0.31	0.70	0.53	0.30	0.93	0.87	0.60
Zane	3.9	17.9	38.5	0.84	0.82	0.43	1.08	0.83	0.36	0.81	0.78	0.67
Forrest	4.3	21.0	37.9	1.13	0.91	0.45	0.83	0.43	0.32	1.00	0.89	0.60
Essex	8.1	22.3	38.5	0.83	0.57	0.37	0.74	0.45	0.33	0.86	0.73	0.62
Cutler 71	9.3	24.6	35.1	0.81	0.64	0.36	0.62	0.49	0.38	0.88	0.84	0.63
Douglas	8.7	26.5	37.2	0.72	0.43	0.21	0.62	0.35	0.21	0.86	0.69	0.52
Toano	10.1	26.7	40.8	1.01	0.62	0.26	0.89	0.52	0.35	0.88	0.73	0.57
Stafford	13.1	27.9	35.7	0.77	0.54	0.24	0.67	0.64	0.29	0.97	0.71	0.51
Hay	9.5	28.6	33.8	1.19	0.74	0.37	0.95	0.46	-.32	0.93	0.80	0.61
Williams82	7.8	29.7	39.3	0.89	0.53	0.46	0.77	0.33	0.29	0.87	0.75	0.62
Pyramid	10.1	32.6	39.9	1.05	0.48	0.15	0.86	0.35	0.22	0.89	0.66	0.36
Regal	11.5	36.2	37.8	0.71	0.46	0.37	0.62	0.35	0.33	0.89	0.73	0.65
MD71-583	9.6	37.9	35.7	0.79	0.60	0.40	0.68	0.45	0.35	0.95	0.74	0.67
Jackson	9.9	39.2	29.2	0.93	0.64	0.38	0.87	0.44	0.27	0.99	0.86	0.69
Ripley	11.6	39.2	32.7	0.85	0.48	0.34	0.92	0.86	0.36	0.91	0.75	0.59
Clark	11.1	44.7	41.5	0.73	0.52	0.32	0.58	0.28	0.22	0.91	0.77	0.64
LSD(0.05)	9.1	9.1	9.1									

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
Institute of Agriculture and Center for Legume Research
Knoxville, TN 37901-1071

Arbitrary Mini-hairpin Oligonucleotide Primers: a tool for analysis of genomes, cloned DNA and PCR amplified fragments

Arbitrary oligonucleotide primers have been used to initiate DNA polymerase-mediated amplification of discrete portions of a target nucleic acid molecule to produce characteristic DNA profiles (Williams et al., 1990; Welsh and McClelland, 1990; Caetano-Anollés et al., 1991). The strategy, collectively termed multiple arbitrary amplicon profiling (MAAP) (Caetano-Anollés et al., 1992a), can identify and isolate molecular markers for genome mapping and general DNA fingerprinting. DNA amplification fingerprinting (DAF)(Caetano-Anollés et al., 1991) uses primers as short as 5 nucleotides (nt) in length and generates very complex amplification patterns. DAF can be used in conjunction with restriction endonuclease digestion of template DNA to enhance detection of polymorphic DNA (Caetano-Anollés et al., 1993); this allowed separation of isogenic lines and identification of markers tightly linked to the supernodulation nts locus in soybean. The use of very short oligonucleotides as primers has permitted study of primer-template interactions with the goal of understanding primer annealing during DNA amplification (Caetano-Anollés et al., 1992b). DAF defined several domains in the primer-template duplex. For example, the first 8 nt from the 3' terminus of the primer were largely responsible for directing the amplification reaction while a 3'-terminal "core" of 5 nt was absolutely necessary for amplification.

Some short oligonucleotides can form extraordinarily stable hairpin structures, consisting of a loop of 3 to 4 nt and a stem of only 2 terminal nucleotides (Hirao et al., 1992). These mini-hairpin oligonucleotides have a stable compact structure and therefore very high melting temperatures and unusually rapid mobilities during electrophoresis in polyacrylamide gels. In this study, we have used extraordinarily stable mini-hairpin oligonucleotide primers harboring a "core" arbitrary sequence at the 3' terminus to prime the amplification of a wide range of templates ranging from plasmid DNA to plant and animal genomes. We discovered that these mini-hairpin primers can have a 3 nt core and still produce complex DNA fingerprints. These primers further our understanding of primer-template interactions during DNA amplification, and provide a

new tool for the analysis of genomes and subgenomic fragments like yeast artificial chromosomes (YACs), cloned DNA and PCR amplified fragments.

Materials and Methods: DNA from indonesian fruit bat, soybean, centipedegrass, and from bacteria was isolated using established protocols. DNA from bacteriophage l cI857ind1Sam7 was obtained from New England Biolabs (Beverley, MA), plasmid pUC18 from Pharmacia (Piscataway, NJ), and plasmid pBR322 from Promega (Madison, WI). Soybean YAC clones were obtained by R. Funke (Plant Molecular Genetics, Knoxville).

DAF reactions (20-25 µl) contained 3 µM primer (synthesized with >99% efficiency), 0.3 units/µl AmpliTaq Stoffel fragment DNA polymerase (PE-Cetus, Norwalk, CT), 200 µM of each deoxynucleoside triphosphates, 4 mM $MgSO_4$, 10 mM KCl, 4 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 20 mM Tris·HCl (pH 8.3), and 0.1 to 5 ng/µl of template DNA. The mixture was overlaid with two drops of mineral oil and amplified in a recirculating hot-air thermocycler for 35 cycles of 30 s at 96°C, 30 s at 30°C, and 30 s at 72°C. Amplification products were separated in polyester-backed 5% polyacrylamide-7M urea slab mini-gels as described (Caetano-Anollés and Bassam, 1993). Electrophoretic mobility of oligomers was analyzed in 20% polyacrylamide gels (Hirao et al., 1992). DNA was detected at the picogram level by silver staining (Bassam et al., 1991) according to a slightly modified protocol (Bassam and Caetano-Anollés, 1993). Backed gels were preserved by drying at room temperature. Silver stained DNA profiles were analyzed with an enhanced laser densitometer (Ultrascan XL, LKB-Pharmacia, Bromma, Sweden) and evaluated using the GelScan XL version 2.1 program (Pharmacia).

DNA amplifications were simulated using the Lightspeed Pascal program Amplify (W. Engels, Genetic Department, University of Wisconsin, Madison, WI) for the Macintosh computer (Apple, Cupertino, CA). The target sequence was searched in both directions for matches to primer sequences selected according to various parameters determining how likely a given match is to contribute to the amplification reaction. The program identifies possible amplification products and establishes which of them are more likely to be amplified based on length, sequence, GC content, and other parameters.

Results and Discussion: Primers were designed by attaching a 7-8 nt mini-hairpin structure to the 5' end of a "core" oligonucleotide of arbitrary sequence (Fig. 1).

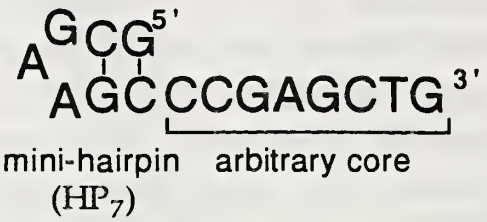


Figure 1. Mini-hairpin oligonucleotide primer harboring an 8 nt arbitrary "core".

DNA from low and high complexity genomes, ranging from bacterial plasmids and bacteriophage lambda to soybean and indonesian fruit bat. DNA fingerprints were complex (with about 10-50 bands in the 50-1000 nt size range) and highly reproducible. Optimal amplification required 3-6 mM $MgSO_4$ concentrations, a primer concentration of 3 μM for complex genomes or 30 μM for PCR fragments, plasmids or lambda phage, and a minimum of 1 ng/μl or 0.1 ng/μl of template DNA from low or high complexity genomes, respectively.

Mini-hairpin primers of decreasing length (derived from the sequence shown in Fig. 1) were designed by removing nucleotides from the 5' end of the arbitrary core, and used to direct amplification of animal, plant, bacterial and viral DNA. While the presence of the hairpin at the 5' terminus of the octamer resulted in pattern variation, many products were common and expressed the expected mobility shift towards higher molecular weight. Results suggest that the mini-hairpin is somehow involved in the formation of many amplification products, and are consistent with our previous observation that overhangs in both strands of a primer-template duplex influence DNA amplification (Caetano-Anollés et al., 1992b).

Decreasing the length of the 8-nt core resulted in highly variant DNA patterns, suggesting that only a fraction of annealing sites are well amplified and become amplicons. The core sequence could be decreased in length to 3 nt and still allow production of reproducible fingerprints. Shorter core regions either produced no or very few amplification products. DNA amplification using primers with only 3-nt arbitrary domains was especially surprising, because the hairpin sequence *per se* failed to amplify DNA to detectable levels. This indicates that the active annealing sequence is that of the 3' terminus, and suggests that a 3-nt sequence can still discriminate between

annealing sites and be extended with considerably efficiency by DNA polymerase in the presence of a quasi-neutral hairpin extension tail.

We amplified DNA from the circular plasmids pUC18 and pBR322 with mini-hairpin primer HP_7-CTG, predicted those sequences that were to be amplified with high probability, assigned actual amplification products to them, and confirmed assignment by restriction endonuclease digestion of amplification products. Amplicons were defined by 13 of 18 possible annealing sites that involved 5-8 nt of primer sequence. Almost all products resulted from perfect annealing of the the arbitrary core and imperfect annealing of either the 3' palindrome or the loop of the mini-hairpin. The fact that the 5' palindromic sequence of the mini-hairpin is not involved suggests that the hairpin structure remains in tight conformation during primer annealing. Results also show that preferential amplification of particular amplicons is strongly influenced by the nature of the annealing interactions established at the amplicon termini. Hairpin-looped template strands appear to serve as preferential sites for annealing during the second round of amplification.

We used mini-hairpin primers to analyze established varieties centipedegrass. DAF analysis of five centipedegrass cultivars ('Tennessee Hardy', 'Tennessee Tuff', 'Oklawn', Centennial', and 'Tifton common') with 8 short mini-hairpin primers revealed a total of 245 amplification products (≤700 bp), 24% of which were polymorphic and generated by every primers. In contrast, DAF using 14 arbitrary octamers produced 221 products, only 14% of which were polymorphic and generated by only 4 primers (Weaver, 1993). Results show that mini-hairpin primers can increase detection of polymorphic DNA.

We also used mini-hairpin primers with short arbitrary cores to analyze low complexity genomes and small DNA molecules. Specific fingerprints were obtained when analyzing yeast artificial chromosomes (YACs) containing 100-330 kb of cloned soybean DNA. In these studies, YAC-characteristic fingerprints were produced despite the presence of common yeast genomic DNA background. Analysis of cloned DNA with mini-hairpin primers could conceivably confirm identification of overlaps in YACs and cosmid libraries for genome analysis. Consistent DAF profiles were also generated from a 1.1 kb PCR product defining a sequence-tagged site (STS) within pUTG-132a, a marker tightly linked to the *nts* locus in soybean (Landau-Ellis et al., 1991), and used to distinguish several soybean experimental lines.

MAAP techniques have been used extensively in breeding and general DNA fingerprinting of a wide range of organisms. We here present a new MAAP-derived fingerprinting tool that increases detection of polymorphic DNA and extends amplification analysis to small segments of DNA. DAF using mini-hairpin primers allow for a more controlled amplification reaction and the possibility of synthesis of closed sets of primers where all possible sequence-variants can be explored. We envision the use of these primers in routine and fast DNA fingerprinting of anonymous templates of wide origin, either directly or through analysis of PCR amplified sequences.

References:

Bassam, B. J., G. Caetano-Anollés, G. 1993. Silver staining of DNA in polyacrylamide gels. Appl. Biochem. Biotechnol. 42:181-188.

Bassam, B. J., G. Caetano-Anollés, P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-83.

Caetano-Anollés, G. 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Applic. 3:85-94.

Caetano-Anollés, G. B. J. Bassam. 1993. DNA amplification fingerprinting using arbitrary oligonucleotide primers. Appl. Biochem. Biotechnol. 42:189-200.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1991. DNA amplification fingerprinting using short arbitrary oligonucleotide primers. Bio/Technology 9:553-557.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1992a. DNA fingerprinting: MAAPing out a RAPD redefinition. Bio/Technology 10:937.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1992b. Primer-template interactions during DNA amplification fingerprinting with single arbitrary oligonucleotides. Mol. Gen. Genet. 235:157-165.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1993. Enhanced detection of polymorphic DNA by multiple arbitrary amplicon profiling of endonuclease digested DNA: Identifiction of markers linked to the supernodulation locus in soybean. Mol. Gen. Genet. 241:57-64.

Hirao, I., Y. Nishimura, Y. Tagawa, K. Watanabe, K. Miura. 1992. Extraordinarily stable mini-hairpins: electrophoretical and thermal properties of the various sequence variants of d(GCGAAAGC) and their effect on DNA sequencing. Nucleic Acids Res. 20:3891-3896.

Landau-Ellis, D., S. Angermüller, R. Shoemaker, P. M. Gresshoff. 1991. The genetic locus controlling supernodulation in soybean (*Glycine max* L.) co-segregates tightly with a cloned molecular marker. Mol. Gen. Genet. 228:221-226.

Weaver, K.R. 1993. DNA amplification fingerprinting of centipedegrass. M.Sc. Thesis. University of Tennessee. Knoxville.

Welsh, J., M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

Williams, J. G. K., A. R. Kubelik, K. J., Livak, J. A. Rafalski, S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Gustavo Caetano-Anollés & Peter M. Gresshoff

(1) UNIVERSITY OF TENNESSEE
Plant Molecular Genetics & Center for Legume Research
Knoxville, TN 37901-1071
and
(2) NATIONAL UNIVERSITY OF MEXICO (UNAM)
Dept. Plant Molecular Biology, Institute of Biotechnology
Cuernavaca, Mor. Mexico 62271

Isolation and cloning of AFLPs generated by tec-MAAP and tightly linked to the *nts* locus in soybean

Mutational inactivation of the soybean *nts* locus results in profuse root nodulation. This supernodulating phenotype segregates as a single recessive Mendelian trait, and appears conditioned by a shoot factor that normally suppresses the development of *Bradyrhizobium*-induced infections. Similar mutants have been also found in other soybean cultivars and legume species (Caetano-Anollés & Gresshoff 1991, Gresshoff 1993). There is no information, however, on the corresponding gene product nor its direct biochemical function. To analyze those molecular regions of the soybean genome that govern autoregulation of nodulation, we are using marker based approaches (Gresshoff 1993). Several Restriction Fragment Length Polymorphisms located to linkage group H of the USDA-ARS/ISU soybean map (Keim et al. 1990), co-segregated tightly with *nts* in F_2 recombinant populations (Landau-Ellis et al. 1991) and diverse Amplification Fragment Length Polymorphisms generated by DNA Amplification Fingerprinting were found to be linked to the phenotype (Caetano-Anollés et al. 1993). Further development of the 'positional cloning' approach in higher plants, rely on the possibility to convert and use these polymorphisms as sequenced genetic markers (v. gr. STS, Olson et al. 1989; SCARs, Paran & Michelmore 1993), to locate important genes in current maps and by allowing their subsequent capture from cloned DNA fragments. In this project, *nts*-linked AFLPs generated by template endonuclease-cleaved Multiple Arbitrary Amplicon Profiling (tec-MAAP) are used as a suitable way to isolate useful markers for high-resolution linkage and physical mapping of the *nts* locus and other soybean genomic regions.

Material and Methods: Detection of *nts*-linked AFLPs was described earlier (Caetano-Anollés et al. 1993). For tec-MAAP, template soybean DNA from parental, mutant and recombinant (Nts^+) individuals, was restricted with three endonucleases prior to amplification with short (7-8 bp), arbitrary oligonucleotides. Selected

polymorphic bands were cut from dried silver-stained polyacrylamyde gels as described (Weaver et al. 1994); isolated bands were re-amplified in a 20 µL volume containing 3 µM octamer primer, 200 µM each dNTP, 0.1-0.2 units/µL *Thermus aquaticus* Amplitaq Stoffel fragment DNA polymerase (Perkin Elmer/Cetus), 1.5 mM $MgSO_4$, 4 mM $(NH_4)_2SO_4$, 0.1 % Triton X-100, 10 mM KCl and 20 mM Tris-HCl pH 8.3. Thermocycling included 35 cycles of 30 sec/ 96° C; 30 sec/ 30 [or 50]° C and 30 sec/ 72 ° C. For the purification of each band, particular reamplification steps were repeated. Enriched polymorphic fragments were further purified by agarose and cloned into appropriate vectors for Southern hybridization analysis and sequencing.

Results and Discussion: Segregation analysis of various AFLPs between parental and mutants in limited recombinant populations revealed several tightly-linked markers to the *nts* locus (Caetano-Anollés et al. 1993). A group of 15 AFLP from distinctive categories and displaying high association (≤100%) or total repulsion (≈0%) were selected (Table I). Fragments required 4-6 successive re-amplifications to become relatively enriched and suitable to be cloned into plasmid vectors. Confirmation of their identity and polymorphic nature is under way. Also, hybridization patterns are being analyzed to know the abundance and size of genomic regions revealed by these markers.

Purification of tec-MAAP products by reamplification of silver-stained polyacrylamide isolated bands is accelerating the analysis of AFLPs useful for genetic studies in our lab; this could help in cloning similar markers generated by MAAP-related techniques. Considering both the known and proposed mechanisms by which these AFLPs were generated (Caetano-Anollés et al. 1993), this study may show whether these polymorphisms are related to the mutation itself, or if DNA rearrangements close to the *nts* locus occurred. It is also likely that these markers may be represented by, or contained in, repetitive genomic sequences. For those finally resulting as unique or low-copy markers, co-inheritance studies in extended F_2 populations with specific PCR primers, will determine their utility as STS/SCARs to obtain high-density maps that will saturate with markers the *nts* region.

Table I. Isolation of *nts*-linked Amplification Fragment Length Polymorphisms using tec-MAAP†

AFLP No.	Code	Primer used	Segregation Pattern	% in F_2	≈MW (bp)
1	D86L-1	GTAACCCC	1100-1	100ab	225
2	D87K-4	CCTGGTCG		100ab	450
3	D87K-4b			100ab	380
4	D87K-7			100a	105
5	D87K-7b			100a	175
6	D86F-1	GATCGCAG	1100-1	100a	180
7	D86F-2			100a	210
8	D86F-3			100a	350
9	D87M-1	CAGCTCGG	1100-1	100a	450
10	H86E-1	GACGTAGG	0001-1	100a	270
11	F86E-1		0010-0	0a	200
12	K86I-2	GTTACGCC	1110-1	100a	260
13	B86H+77A	GAAACGCC + CGAGCTG	0011-0	0a	220
14	C87I-1	CCTGCTGG	1100-0	0ab	235
15	C86D-1	GTAACGCC	1100-0	0a	230

† Twelve categories of AFLPs were defined according to their presence (1) or absence (0) in *Glycine soja* PI468.397 *G. max* cv. Bragg, allelic mutants *nts382* and *nts1007* derived from cv. Bragg and supernodulating individuals from segregating F_2 populations: a= *G. soja* x *nts1007* [15 plants]; b= *G. soja* x *nts382* [20 plants] (Caetano-Anollés et al. 1993).

Further analysis using these sequences will allow to co-localize linked markers in single PFGE fragments in order to accelerate the selection of genomic YAC clones for positional cloning of the *nts* locus.

References:

Caetano-Anollés, G. and P.M. Gresshoff. 1991. Plant genetic control of nodulation. Annu. Rev. Microbiol. 45:345-382.

Caetano-Anollés, G., B.J. Bassam and P.M. Gresshoff. 1993. Enhanced detection of polymorphic DNA by multiple arbitrary amplicon profiling of endonuclease digested DNA: identification of markers tightly linked to the supernodulation locus in soybean. Mol. Gen. Genet. 241: 57-64.

Gresshoff, P.M. 1993. Molecular genetic analysis of nodulation genes in Soybean *In*: Plant Breeding Reviews (ed. J. Janick), 11: 275-318.

Keim, P., B.W. Diers, T.C. Olson and R.C. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735-742.

Landau-Ellis, D., S. Angermüller, R. Shoemaker and P.M. Gresshoff. 1991. The genetic locus controlling supernodulation in soybean (*Glycine max* L.) co-segregates tightly with a cloned molecular marker. Mol. Gen. Genet. 228: 221-226.

Olson, M., L. Hood, C. Cantor and D. Botstien. 1989. A common lenguage for physical mapping of the human geneome. Science 245:1434-1435.
Paran, I. and R.W. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85:985-993.
Weaver, K.R., G. Caetano-Anollés, P.M. Gresshoff and L.M. Callahan. 1994. Isolation and cloning of DNA amplificatiuon produccts from silver-stained polyacrylamide gels. Biotechniques 16:226-227.

Jaime E. Padilla (1,2)
Gustavo Caetano-Anollés (1)
Peter M. Gresshoff (1)

Index of authors